# CELLULAR ANTIGENS IN SPECIES AND SPECIES HYBRIDS IN DUCKS[1]

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## INTRODUCTION

COMPARISONS of species and species hybrids by means of morphological and anatomical characters have been of interest to biologists for many years. Following the discovery of immunological techniques, applied first principally in bacteriology, differences in serum proteins between related species were demonstrated by many investigators, especially by NUTTALL (1904) followed, among others, by BOYDEN (1926), BAIER (1933), SASAKI (1937), and WOLFE (1939). Differentiation of species by means of antigenic characters in the red blood cells was demonstrated by LANDSTEINER and VAN DER SCHEER in 1924. Using anti-erythrocyte serum and employing the agglutinin-absorption technique, these workers could distinguish between the red blood cells of the horse, donkey, and mule. Previous to this time, however, individual differences in the cellular characters within a species had been demonstrated by LANDSTEINER (1900, 1901), EHRLICH and MORGENROTH (1900), and TODD and WHITE (1910).

Evidence has been presented by IRWIN (1932, 1938, 1939) and by IRWIN and associates (1936a, 1936b, 1936, 1940) that the antigenic components which differentiate the red blood cells of several different species of doves and pigeons are multiple. When species hybrids were mated with either parental species and the backcross hybrids successively mated with representatives of the same parental species for several generations, the antigenic complex which differentiated the species hybrids from the parental species (to which the backcross hybrids were mated) became divided or segregated. The cells of various backcross progeny contained either different parts, combinations of parts, or no part of the original complex. These findings strongly indicate that the individual parts (or units) of the several different species-specific complexes examined are gene controlled.

Hybrids from the mating of Muscovy (*Cairina moschata*) × Mallard (*Anas Platyrhynchos platyrhynchos*), or its domesticated derivatives, have been obtained by a number of investigators. SOKOLOWSKAJA (1935a, 1935b, 1936) observed hybrids from the reciprocal matings of Muscovy × Khaki-Campbell and reported hereditary differences between the species for several morphological characters, some of which were in part controlled by sex-linked genes.

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She also noted differences in the serum proteins between the two species and the species hybrids, using the precipitin test, and suggested that the serum of the hybrids had "some new properties . . . not present in either of the parents." CREW and KOLLER (1936) studied the gonads of the hybrids resulting from the reciprocal matings of Muscovy×Aylesbury, seeking to find a cause of the sterility in the hybrids. They suggested that the sterility was due to complementary genetic factors affecting the relationship between chromosome pairing and spindle development. Reciprocal hybrids resulting from the matings of Muscovy×Pekin have been used by GORDON (1938) in a study of the antigenic differences in their red blood cells with respect to the species-specific and common substances of the parental species. He reported that at least seven different antigenic characters specific to Muscovy and an equal number specific to Pekin were necessary to account for the various combinations of each specific complex encountered in the cells of the hybrids. He also reported the presence of a "hybrid" substance in the corpuscles of all hybrids examined which was not found in the cells of either parental species.

Further study of the antigenic characters in the blood of this species hybrid follow.

## MATERIAL AND METHODS

Hybrids were produced from reciprocal matings of Mallards and Muscovies. The mating of two Muscovy males with five Mallard females produced 33 hybrid offspring, and 39 hybrids resulted from matings of three Mallard males with five Muscovy females.

Immune sera were produced by injecting rabbits intravenously with washed red blood cells, also by injecting ducks intravenously with citrated whole blood. For the rabbit injections, three ducks (A596, P691, and P694), each of which had produced hybrids, served as the donors of cells of Muscovy; the cells of Mallard were obtained from three ducks (A635, 964, and 967) which also had hybrid progeny. The $F_1$ cells were obtained from two sibs from the mating of a Muscovy male with a Mallard female. Each rabbit received an initial injection of 2.0 cc of a 25 percent suspension of pooled cells. The second and subsequent 20 injections were given daily after the second day and each consisted of 1.0 cc of a 10 percent suspension of washed cells. The rabbits were bled on the third and fourth days following the last injection of cells. The blood was allowed to clot and the serum was pipetted off. The serum was stored in the icebox with merthiolate added as preservative.

For the immune sera developed in ducks, individual hybrids served as donors. Prior to the first injection of cells into the ducks, a sample of the recipient's serum was always mixed with selected cells of Mallard, Muscovy, and $F_1$ to test for the presence of normal antibodies. No agglutination of any cells used was ever observed in the serum of 32 different hybrids used in immunization. The immunization procedure with ducks consisted of an initial injection of 10.0 cc of citrated blood, then 5.0 cc seven days later, and five subsequent injections of 5.0 cc given at four day intervals. Trial bleedings were

generally made beginning the second week, just preceding each injection. Those individuals showing good antibody response were bled on two consecutive days (3–6) following the last injection of cells.

Agglutinations were performed by adding to 0.1 cc of immune serum in varying dilutions (increasing by halves) one drop of a 2.0 percent saline suspension of washed red blood cells. The titer of an immune serum was the highest dilution at which agglutination was observed macroscopically. For the absorptions, the immune serum was diluted according to its titer and mixed with a quantity (usually one-half volume) of washed cells. The mixture was agitated gently and allowed to stand at room temperature (25°C) for 15–20 minutes (twice this period when immune serum produced in ducks was used). This procedure was repeated several times until the fluid no longer agglutinated the cells used in absorption. Each subsequent absorption was allowed to stand for two hours and usually at least one was completed at icebox temperature overnight. Readings of agglutination were generally made after two hours at room temperature, and again after storage overnight in the icebox. Tubes showing no agglutination or faint reactions were usually checked microscopically.

## RESULTS

### *Differentiation of the species and species hybrids*

The reactions by which the cells of Muscovy, Mallard, and the $F_1$ may be distinguished are given in table 1. Direct agglutination tests with each immune serum failed to differentiate the three kinds of cells. However, when anti-

TABLE 1

*Serological relationships between the red blood cells of Muscovies, Mallards, and their hybrids.*

| IMMUNE SERUM | ABSORBED BY CELLS OF | FIRST SERUM DILUTION | AGGLUTINATIONS WITH CELLS OF | | | |
|---|---|---|---|---|---|---|
| | | | MUSCOVY | MALLARD | MUSCOVY ♂× MALLARD ♀ | MALLARD ♂× MUSCOVY ♀ |
| Muscovy | — | 1:30 | 10, 11 | 9, 10, 11 | 10 | 10 |
| Muscovy | Mallard | 1:30 | 7, 8 | 0 | 6, 7 | 6, 7 |
| Mallard | — | 1:30 | 10, 12 | 10, 12 | 10 | 10 |
| | Muscovy | 1:15 | 0 | 7, 8 | 6, 7, 8 | 6, 7, 8 |
| $F_1$ (Muscovy ♂× Mallard ♀) | — | 1:15 | 11 | 10, 11 | 11 | 11 |
| | Mallard | 1:15 | 6 | 0 | 6 | — |
| | Mallard & Muscovy | 1:15 | 0 | 0 | + | + |

The digits in this and in following tables refer to the last dilution at which agglutination was observed macroscopically—that is, 1=30, 2=60, 3=120 . . . 10=15,360. Symbols: ++= strong agglutination, +=definite agglutination, ±=definite agglutination though clumps smaller, 0=no agglutination; at the first (absorbing) dilution of the antiserum. A dash (—) indicates that the absorption or combination was not made.

Muscovy serum (developed in rabbits) was absorbed by the cells of Mallard until no agglutination occurred with these cells at the absorbing dilution, the cells of both Muscovy and the $F_1$ were agglutinated, but at a lower dilution (7th or 8th corresponding to one part of immune serum in 1920, or in 3840 parts of saline) than was observed prior to the absorption (10th or 11th). This fluid constituted a "reagent," in that it contained antibodies which clumped only cells of Muscovy or the $F_1$ by virtue of specific substances of Muscovy not present in the cells of Mallard (antibodies for substances *common* to the two species are removed from the immune serum by the cells of Mallard). Similarly when anti-Mallard serum was completely absorbed by the cells of Muscovy, a reagent was developed for substances of Mallard not shared with Muscovy. This reagent agglutinated cells of both Mallard . nd the $F_1$ at comparable dilutions, but not those of Muscovy. The reactions of $F_1$ cells to these two reagents indicated that at least part of the antigens specific to Muscovy and to Mallard, respectively, were contained in the cells of the species hybrids. Likewise, when anti-$F_1$ (Muscovy ♂ × Mallard ♀) serum was absorbed by the cells of Mallard, antibodies remained in the serum which clumped the cells of the hybrids and Muscovy. Furthermore, when anti-$F_1$ serum was absorbed by the cells of both parental species (including the actual parents of the hybrids) antibodies remained which agglutinated the cells of each $F_1$ tested [13 $F_1$ (Muscovy ♂ × Mallard ♀) and 3 $F_1$ (Mallard ♂ × Muscovy ♀)]. This test constituted evidence for the presence of a new or "hybrid" substance in the cells of the hybrids, not present in either parent.

A diagrammatic representation of the antigenic components in the red blood cells of the two species and their hybrid would resemble that depicted by IRWIN and COLE (1936a) for two species of doves (Pearlneck and Ringdove) and their hybrid. The proportions of the common and species-specific components, however, would probably be different in a comparison of these species of ducks.

If one were given three samples of blood, labeled A, B, and C, known to be from each species and their hybrid, it would be relatively easy to identify each of the samples by virtue of the cell reactions with the three reagents mentioned above, that is, for (1) Mallard specific, (2) Muscovy specific, and (3) the hybrid substance. If the cells from sample "A" were agglutinated by each of the three reagents, then that sample was from a hybrid; if cells from sample "B" were clumped by reagent #2 but showed no agglutination with reagents #1 and #3, then it was from a Muscovy; and similarly if those of sample "C" were agglutinated by reagent #1 and showed no agglutination with #2 or #3, then sample "C" was from a Mallard.

In summary, it may be stated that the corpuscles of Muscovy contain substances specific to itself and others shared with Mallard. The cells of Mallard likewise contain antigens peculiar to itself as well as others held in common with Muscovy. The species hybrids, on the other hand, except for individual differences in the three kinds of characters, contain at least a part of the antigens specific to Muscovy and to Mallard, respectively, and all the cellular

characters common to both parental species. In addition, a component not found in either parental species was demonstrable in the cells of the hybrids, presumably as a result of the interaction of genes which produced species-specific constituents in each species.

*Differences between hybrids in cellular antigens specific to Muscovy*

Tests for differences between species hybrids in their content of antigens specific to Muscovy, which, if found, would imply heterozygosity of the Muscovy parent for genes controlling these characters, were made as follows. As described above, anti-Muscovy serum when completely absorbed by the cells of Mallard provided a reagent specific for those substances present only in the corpuscles of Muscovy. Should there be reactions with the cells of other hybrids using a reagent prepared by absorbing Muscovy anti-serum with the cells of both Mallard and a species hybrid, either in combination or in successive absorptions, they would denote differences between the hybrids in cellular antigens specific to Muscovy.

A summary of the interactions between selected reagents of this kind and the cells of representative hybrids is given in table 2. Controls for the completeness of absorption were provided by testing each reagent with the cells of Mallard

TABLE 2

*Tests for individual differences between species hybrids in content of specific Muscovy substances in their cells.*

| CELLS OF | ANTIGENS PROPOSED | IMMUNE SERUM OF MUSCOVY FIRST ABSORBED BY MALLARD CELLS THEN BY THE CELLS OF EACH OF THE $F_1$ BIRDS LISTED BELOW. SERUM DILUTION 1:15 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 122 | 739 | 740 | 845 | 775 | 810 | 118 | 119 | 739 + 740 |
| Muscovy | A-1, A-2, B, C, D, E | − | + | ++ | ++ | ++ | ++ | ++ | ++ | − |
| Mallard | — | o | o | o | o | o | o | o | o | o |
| P694♂ × 122 | A-1, E | o | + | o | + | + | + | + | + | o |
| P694♂ × 739 | A-2, E | + | o | + | o | ++ | + | + | + | o |
| P694♂ × 740 | A-1, E | o | + | o | + | ++ | + | + | + | o |
| P694♂ × 845 | A-2, E | ± | o | + | o | + | + | + | + | o |
| A596♀ × 775 | B, C | + | + | + | ++ | o | + | + | + | + |
| A596♀ × 810 | B | ± | + | + | + | o | o | + | + | + |
| P691♂ × 118 | D | + | + | + | + | + | + | o | o | + |
| P691♂ × 119 | D | ± | + | + | + | + | + | o | o | ± |
| P694 | A-1, A-2, E | ± | + | ± | ++ | + | ++ | − | − | − |
| A596 | B, C | ++ | + | + | ++ | + | + | − | ++ | ++ |
| P691 | D | ++ | − | − | − | − | − | − | − | − |
| Column | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |

For explanation of symbols, see table 1.

and the individual hybrid used; tests with the cells of Muscovy served as the positive control.

*Contrasting antigens A-1 and A-2*

A definite segregation of cellular characters specific to Muscovy was found among 25 species hybrids resulting from the mating of a Muscovy male (P694) with three Mallard females. The interactions of the cells of four hybrids (122, 739, 740, and 845) with reagents prepared by using their cells, and those of hybrids from other matings, separately in absorptions of anti-Muscovy serum are presented in table 2. It may be stated that for any immune serum, the cells of two or more hybrids whose antigens were equivalent should give identical reactions with reagents prepared by using other cells in absorption. Their respective reagents should also react alike with all cells tested. This criterion for the reactivity of equivalent cells has been observed in each instance where two or more cells are considered identical in antigenic composition.

It will be noted that the cells of hybrids 122 and 740 removed by absorption the antibodies for each other, but the reagents so produced agglutinated the cells of both 739 and 845. Furthermore, within this family there were 11 other hybrids (not listed in the table) whose cells by absorption removed antibodies for the cells of hybrids 122 and 740 as well as for each other, but these reagents agglutinated the cells of hybrids 739 and 845. The cells of these 11 hybrids were indistinguishable from and therefore presumably identical with those of hybrids 122 and 740 in content of antigens peculiar to Muscovy. Similarly the cells of either 739, 845, or each of ten other hybrids (not listed in the table) of the offspring of this Muscovy male (P694) by absorption in combination with cells of Mallard removed antibodies for each other but not for any of the hybrids identical with 122 and 740. From this evidence it is proposed that the cells of hybrids 122 and 740 and the 11 others contain an antigen called "A-1," and those of the 12 other hybrids of the family (including 739 and 845) contain a different and contrasting antigen designated "A-2."

On a genetic basis, these results are most logically interpreted by assuming that the Muscovy parent (P694) was heterozygous for a single pair of chromosomes carrying one or more genes producing antigens A-1 and A-2, respectively, peculiar to Muscovy. That is, P694 was heterozygous for genes affecting A-1 and A-2, and homozygous for all others producing those Muscovy specific characters which were detected by the antisera used. The chances of such a distribution of characters being the result of segregation of genes on more than one pair of chromosomes is very remote (less than 1 in 10,000). Hence a single pair of chromosomes is assumed to carry the gene or genes responsible for the contrasting antigens A-1 and A-2.

However, it will be noted that these two kinds of reagents agglutinated the cells of all hybrids tested from other matings (columns 3, 4, 5, and 6, table 2). That is, antibodies for specific Muscovy characters, other than those carried by the two kinds of hybrids in the family of the Muscovy (P694), must have remained in the serum following these absorptions. This proposal is further

substantiated by the observation that the cells of these hybrids (from other matings) and Muscovies were still reactive with the fluid following the double absorption with cells containing A-1 and A-2 (see column 11, table 2). Such antigens, different from those of the Muscovy (P694) and therefore from its hybrids, must have been present in the cells of hybrids from the other families.

### *Antigens B and C*

Other reagents were prepared from anti-Muscovy serum by absorbing with cells from each of five hybrids resulting from the mating of a female Muscovy (A596) with a Mallard. The interactions of two such reagents with representative cells are also given in table 2. The reagent prepared by using in absorption the cells of hybrid 775 (column 7) failed to agglutinate the cells of the four other sibs, as did also the reagents prepared by using the cells of two other hybrids of this family not listed in the table. These three hybrids were indistinguishable and therefore presumably identical in their content of Muscovy specific substances, and each contained all such substances possessed by any hybrid of this family. However, when the cells of hybrid 810 (column 8) or one other hybrid (not listed) were used in absorption of Muscovy antiserum, each removed agglutinins for the cells of the other, but the reagents so produced agglutinated the cells of hybrid 775 and of the two hybrids identical with it. The cells of 810 and the other hybrid were therefore presumably equivalent in their content of specific Muscovy antigens, but each contained only a part of the antigens of hybrid 775. Thus the hybrids of the Muscovy female (A596) may be classified in two groups comprising (1) three hybrids, including 775, whose cells contain all the specific Muscovy substances contributed by their Muscovy parent to any of the hybrid offspring and (2) two other hybrids including 810, whose cells contain only a part of these Muscovy characters.

As a result of these reactions, together with the finding that the cells of all hybrids from this family were agglutinated by all other reagents (prepared by using the cells of hybrids of other families in absorption), it is proposed that the cells of the three hybrids of the first group (including hybrid 775) contain a minimum of two antigens designated B and C, each different from A-1 and A-2, and that the cells of the two individuals (hybrid 810 and the other) in the second group contain only one of these antigens, as B.

### *Antigen "D"*

When the cells of two other hybrids (118 and 119), resulting from the mating of a Muscovy male (P691) with Mallard females, were employed in absorptions of Muscovy antiserum in combination with the cells of Mallard, each removed agglutinins for the cells of the other (table 2, columns 9 and 10), and, since the cells of these two hybrids gave parallel reactions with the other reagents listed in the table, they are indistinguishable and may be assumed to be identical in antigens specific to Muscovy. The cells of these hybrids were reactive with the reagents obtained following absorptions of the anti-Muscovy serum with cells containing A-1, A-2, B and C, implying that at least one additional

antigen peculiar to Muscovy was present in their cells. This has been called "D."

*Antigen "E"*

As stated previously, the cells of the two kinds of hybrids (as 122 and 739) from Muscovy P694 possessing antigens A-1 and A-2, repectively, were reactive with the reagents prepared by the absorption of anti-Muscovy serum with cells of hybrids containing antigens B and C together, B, or D. Likewise the cells of hybrids containing B and C (775) or D (118) were agglutinated by the test fluids obtained by exhaustion of the antiserum for antibodies by both A-1 and A-2 cells (table 2, column 11). This indicates that the hybrids from each of the three families possessed at least one antigen peculiar to the particular Muscovy parent. These are exemplified by antigens B and D in the cells of the Muscovies A596 and P691, respectively. In order to account for the reactivity of the cells of hybrids from the Muscovy, P694, to all reagents (save those using cells from identical sibs of this family in absorption), it is assumed that P694 and its hybrids contain at least one additional antigen called "E."

*Further evidence of the existence of the hybrid substance*

As stated earlier, the cells of all hybrids tested posssessed a hybrid substance which was detected by the reagent prepared from anti-$F_1$ serum (developed in rabbits) following absorption by the cells of both parental species, including those of the actual parents.

It has been possible to demonstrate the presence of this hybrid substance by another quite independent method. The bloods of two hybrids chosen at random from a mating were pooled and used to immunize both of the actual Mallard and Muscovy parents. For example, the cells of hybrids, 845 and 116, from the Muscovy male, P694, were used to immunize their Mallard parent, 967, as well as the Muscovy, P694. In the Mallard, antibodies would be expected for those substances which are specific to Muscovy and not shared with Mallard; while in the Muscovy, agglutinins would be anticipated for those Mallard specific characters contained in cells of the hybrids used in immunization. In these antisera, antibodies would also be expected for any substance present in the cells of the hybrids, but not in those of either parent, as the result of the interaction of genes from the two species. Each of the antisera so produced was then absorbed with the cells of the other parent of these hybrids—that is, anti-$F_1$ produced in 967 was absorbed with the cells of the Muscovy, P694, and vice versa. As the result of these absorptions, all antibodies for the Muscovy substances were removed from the antiserum produced in Mallard, 967, as well as all those for Mallard characters from the antiserum obtained from the Muscovy, P694, by the reciprocal absorption. Following these absorptions, antibodies would be expected to remain in each reagent only for those substances in the cells of the hybrids that were not present in those of either parent. Thus there are two antisera possible (each yielding a reagent) for each hybrid family. Six such antisera from three different matings have been tested.

TABLE 3

*Tests for the presence of the "hybrid" substance and its divisibility, using antisera produced in each of the parents of the hybrids.*

| | | ANTI-$F_1$ (845 & 116)* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRODUCED IN P694 (MUSCOVY) | | | | | | PRODUCED IN 967 (MALLARD) | | | | | |
| | | ABSORBED BY: | | | | | | | | | | | |
| CELLS OF: | | — | MALL. (967) | 967 + 670 | AND 844 | 3277 | 131 | — | MUSC. (P694) | A588‡ + 670 | AND 844 | 3277 | 131 |
| Mallard | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Muscovy | | 0† | 0 | 0 | 0 | 0 | 0 | 9, 10 | 0 | 0 | 0 | 0 | 0 |
| P694 ♂ | 670 | 9 | ++ | 0 | 0 | 0 | 0 | 9 | ++ | 0 | 0 | 0 | 0 |
| | 844 | — | ++ | 0 | 0 | 0 | 0 | — | ++ | 0 | 0 | 0 | 0 |
| | 845 | 9 | ++ | 0 | 0 | 0 | 0 | 10 | ++ | 0 | 0 | 0 | 0 |
| | 116 | 9 | ++ | 0 | 0 | 0 | 0 | 8, 9 | ++ | 0 | 0 | 0 | 0 |
| | 3277 | — | + | 0 | 0 | 0 | 0 | — | + | 0 | 0 | 0 | 0 |
| A705 ♀ | 1261 | — | + | 0 | 0 | 0 | 0 | — | + | 0 | 0 | 0 | 0 |
| A704 ♀ | 131 | — | + | 0 | 0 | 0 | 0 | — | + | 0 | 0 | 0 | 0 |
| A587 ♀ | 269 | — | ++ | 0 | 0 | 0 | 0 | — | + | 0 | 0 | 0 | 0 |
| | 271 | — | ++ | 0 | 0 | 0 | — | — | ++ | — | — | 0 | — |
| A588 ♀ | F992 | — | ++ | 0 | 0 | — | — | — | ++ | — | — | — | — |
| Column | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

(See table 1 for explanation of symbols.)

* Serum dilutions, 1:4 for double absorptions; 1:5 elsewhere.

† The cells of two Muscovies were agglutinated by this unabsorbed antiserum.

‡ The cells of A588 were used instead of those of P694, since the two cells were found to be identical by reciprocal absorption tests using this anti-$F_1$ serum from Mallard (967).

The details of tests with two of the antisera so produced are given in table 3. No agglutination was ever observed between the cells of any representative of either parental species and the reagent obtained following the absorption of an anti-$F_1$ serum (produced in ducks) by cells of the particular opposite parent. That is, no reaction occurred between the cells of Mallards or Muscovies and the reagent prepared by absorbing anti-$F_1$ from Muscovy, P694, with cells of Mallard, 967 (column 2), or with that prepared by absorbing the antiserum from Mallard (967) by the Muscovy, P694 (column 8, table 3). The cells of the hybrids, however, were regularly agglutinated by each of these reagents, as shown by the interactions between the cells of ten hybrids from five different matings and these two reagents, in columns 2 and 8 of table 3. Furthermore, the cells of 72 hybrids (all that were produced in this study) have been tested

with at least two of the six reagents developed, and the cells of each were agglutinated. These results indicate that all the hybrids contain at least a part of the hybrid substance in their corpuscles.

A few tests have been made to determine whether the hybrid substance could be fractionated. The cells of individual hybrids chosen at random (always in combination with those of the appropriate parent) were used in absorption of the two antisera listed in table 3. As is shown in the table, the reagents prepared by using the cells of hybrids, 670, 844, 3277, or 131, in absorptions of the anti-$F_1$ serum produced in each parent, failed to agglutinate the cells of any hybrid tested, and obviously none of the parental cells (columns 3, 4, 5, 6, 9, 10, 11, and 12, table 3). This indicated that the cells of each of the four hybrids used in absorptions contained all of the hybrid substance which was possessed by any hybrid whose cells were tested with these reagents, regardless of family origin. That is, the hybrid substance was indivisible by these tests, although these results do not establish that it is a single component.

Evidence was also obtained suggesting that the Muscovies used in this study differed in their complement of antigens shared with Mallard. Limited tests involving some of these substances common to the two species suggested that they are transmitted in Muscovies as genetic characters. No differences were noted among the Mallards tested for these characters; each Mallard seemingly possessed all the common characters for which differences were detected in the Muscovy species. The details of the tests supplying evidence for the above statements are not included in the tables, but will be furnished if requested.

## DISCUSSION

The hybrids from this cross are sterile, hence no corpuscles of the $F_1$'s backcross offspring were available for assay. Differences in antigens specific to Muscovy among the hybrids of a given mating presumably may be detected only if the Muscovy parent were heterozygous for the genes controlling the characters. As has been pointed out by IRWIN and COLE (1936a), the genes producing species-specific characters undoubtedly exist in the $F_1$ in the simplex state, while only those determining the common substances find homologues. Thus, if a Muscovy were heterozygous for a pair of chromosomes controlling species-specific substances, the results of mating this individual with a Mallard would be a distribution of these characters among the hybrid offspring simulating that expected within a species in the backcross of a monohybrid to a recessive.

The six antigens, A-1, A-2, B, C, D, and E, which differentiate the hybrids resulting from matings of Muscovies with Mallards, also denote differences between the cells of the three Muscovy parents of these hybrids. There can be little doubt as to the heritable nature of these antigenic substances. Four of them (A-1, A-2, D, and E) were transmitted to the hybrids by male Muscovies. Furthermore, segregation was observed among the hybrids from the male Muscovy (P694) for the contrasting characters, A-1 and A-2, as well as for

antigen C among the hybrids resulting from the female Muscovy (A596). However, as shown by the cell reactions of the hybrids with the Muscovy specific reagents, no one of the Muscovy parents of the hybrids possessed all these characters. For example, the results of tests with his hybrids show that the Muscovy male, P694, possessed genes for the contrasting characters, A-1 and A-2, and was homozygous for genes for E. This male Muscovy also probably carried alleles for antigens recessive to B, C, and D, or alleles with other effects. At least one gene was present in each of the Muscovy parents which, by interaction with one or more from Mallard, produced the hybrid substance.

Thus there are at least six antigens by which Muscovies may differ when contrasted with Mallard. If these are produced by one or more genes on independent chromosomes (except those for A-1 and A-2 which are on the same pair) there are at least five pairs of chromosomes in Muscovy which make for antigenic dissimilarity between the two species. Probably as many or more pairs of chromosomes are likewise concerned in the production of substances common to the two species. It should not be inferred, however, from the evidence reported that the six characters specific to Muscovy are the only ones making the cells of Muscovy distinct from those of Mallard. There may very well be others.

Since the antigens A-1 and A-2 were identified as contrasting characters, one would expect each of the other Muscovies (A596 and P691) to possess at least one of these characters and their hybrids one or the other. The results of other tests, to be presented in another report, show these contrasting characters are each further divisible into several parts. The possibility of a similar situation prevailing for one or all the other antigens, B, C, D, and E, cannot be excluded.

The hybrid substance was found in the cells of all hybrids produced in this study. It is assumed that this substance is produced in the cells of the hybrids by the interaction of genes which in the parental species determine species-specific characters. It was not possible in this study to fractionate this substance by immunological means. However, it is conceivable that the hybrid character may indeed be a complex conditioned by several genes, implying as many component parts, and that the genes were in the homozygous state in each of the parental species. This would make for identity among all hybrids with respect to this character. Evidence has been presented by IRWIN (1939) showing that the hybrid substance present in the cells of $F_1$ and backcross hybrids from Pearlneck $\times$ Ringdove consists of at least two parts, but appears as a unit when the cells of $F_1$'s are used in absorption of anti-$F_1$ serum, similar to the tests reported here.

There was no difference observed in the reactivity of the cells of hybrids from reciprocal matings to the reagents for the respective species-specific components. In fact, the greater part of the tests performed in this study dealt with the Muscovy specific substances in the cells of hybrids which received the genes for these characters from the male parent. These results indicate there was little, if any, effect of the maternal cytoplasm on these characters.

SUMMARY

By means of immunological techniques, the red blood cells from Mallards, Muscovies, and hybrids between these species have been examined for antigenic similarities and differences. Each species possessed antigens specific to itself as well as others which were shared. The cells of the $F_1$'s examined contained the major part of the antigens specific to each of the parental species and presumably all those common to both. In addition, the hybrids possessed a new or "hybrid" substance which was not found in the cells of either parent. The existence of the hybrid character was demonstrated by two independent methods. It was not possible by the tests employed to fractionate this substance.

Differences in antigens specific to Muscovy were noted among the hybrids from each of two matings. These results would be expected on a genetic basis, by virtue of the segregation of genes controlling those characters which were carried in the heterozygous state by the respective Muscovy parents. For example in one family comprising 25 hybrids from a Muscovy male, the offspring possessed one or the other of two antigens. These contrasting antigens presumably were controlled by one or more genes on a single pair of chromosomes of Muscovy.

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# GROWTH CHANGES IN MAIZE ENDOSPERM ASSOCIATED WITH THE RELOCATION OF CHROMOSOME PARTS

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IN PREVIOUS studies of somatic segregation in relation to atypical growth, various alterations in color and in cellular arrangements in the endosperm of the maize kernel were described. The tissue alterations are seen as depressions and outgrowths on the surface of the mature, dry seed. They vary greatly in size and outline but are usually small and ordinarily cannot be seen readily without the aid of a low power microscope and a good light.

In the earlier reports (JONES 1937, 1940) it was shown that growth changes accompanied a loss or an accumulation of chromosome parts resulting from unequal mitoses. The present study gives additional evidence and shows that the loss and accumulation of genic material which occurs is only a secondary factor. The breaking and relocation of chromosomes at specific places gives an interpretation that is more consistent with all of the facts known at the present time.

## DESCRIPTION OF GROWTH CHANGES

The growth changes may coincide with other endosperm alterations or they may accompany such changes in adjoining areas in a paired arrangement indicating that the alterations started from a single cell during development and that one or more distinct areas resulted from an unequal division of nuclear substances. They also occur without any other visible change on the surface of the seed but are nearly always altered in staining reaction. The outgrowths stain darker with iodine than the surrounding normal tissue, and the depressions stain lighter or not at all. The border line between normal and aberrant endosperm is usually well marked, and the extent of the change can be followed toward the center of the seed by removing successive layers of endosperm tissue with a file and staining each layer.

## GROWTH CHANGES ASSOCIATED WITH CHROMOSOME 5

While growth changes of this kind are associated with all of the easily seen gene markers, it was noted in previous studies that they were somewhat more frequent when markers on the long arm of chromosome 5 were followed. The two most convenient markers on this chromosome are brittle endosperm-1 (*bt Bt*) and red aleurone-1 (*pr Pr*).

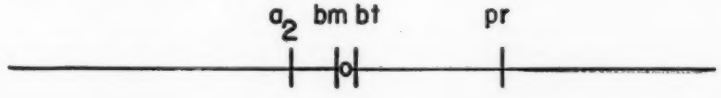


FIG. 1

Brittle endosperm (MANGELSDORF 1926; EMERSON, BEADLE, and FRASER 1935) is a most useful endosperm marker. In heterozygous seeds, removal of the dominant allele results in shrunken areas that are somewhat translucent and glassy in large areas. Small areas are not so easily seen but are clearly delineated by staining with a weak iodine solution. Brittle areas are clearly differentiated from sugary mosaic areas by a lack of swelling that is characteristic of sugary tissue when water is applied. Sugary cells are also more elongated. Both brittle and sugary areas stain more lightly than the surrounding normal tissues.

Red aleurone results from the interaction of several complementary color genes in the absence of dominant *Pr*. In heterozygous mature dark bluish-black kernels the removal of *Pr* shows as clearly distinct red spots that can be differentiated in single cells. These red areas are sometimes paired with areas that are darker than the surrounding normal cells. These dark spots are not so distinct. Their visibility depends upon the depth of color of the normal tissues. If these are dark, additions of *Pr* alleles may make no noticeable increase in color. In medium and lightly colored aleurones they are fairly distinct.

These two genes affecting endosperm texture and aleurone color are located on the long arm of chromosome 5. *Bt* is close to the centromere on the opposite

TABLE 1

*Frequency of Mosaic Areas in Colored Aleurone Seeds Having the Composition bt bt Bt, pr pr Pr.*

| TOTAL NUMBER OF SEEDS | UNPAIRED | | | PAIRED | | | |
|---|---|---|---|---|---|---|---|
| | RED | RED BRITTLE | RED RAISED | RED BRITTLE DARK NORMAL | RED NORMAL DARK NORMAL | RED NORMAL DARK RAISED | RED RAISED DARK NORMAL |
| 641 | 106 | 19 | | 3 | 6 | 1 | |
| 1240 | 81 | 27 | | 1 | | | |
| 882 | 43 | 10 | | 1 | 5 | | |
| 93 | 5 | 2 | | | | | |
| 1649 | 46 | 26 | | 1 | 1 | | |
| 823 | 12 | 9 | | 2 | 3 | | |
| 3553 | 94 | 56 | 5 | | 1 | | 5 |
| 320 | 1 | 1 | | 1 | 1 | | |
| 258 | | 2 | | | | | |
| 703 | 10 | 5 | | 2 | 3 | | |
| 403 | 10 | 3 | | | | | |
| 479 | 8 | 7 | | | 2 | | |
| 974 | 84 | 23 | 2 | | 4 | | 1 |
| 808 | 81 | 13 | | | 3 | | 1 |
| 621 | 59 | 4 | | | 2 | | |
| 1058 | 83 | 7 | 2 | | 1 | 1 | 1 |
| 14505 | 723 | 214 | 9 | 11 | 32 | 2 | 8 |
| per 10 M | 498 | 148 | 6 | 8 | 22 | 1 | 6 |

side from *Bm* and $A_2$ (RHOADES 1936) and *Pr* is somewhere in the central region, about 20 crossover units to the right of *Bt* as shown in figure 1.

## MATERIAL EXAMINED

In table 1 are given the findings from 16 groups of ears resulting from the cross pollination of a few closely related *bt pr* stocks with different *Bt Pr* stocks of widely diverse origin, comprising pop, flint, floury, dent, sugary, and mixtures of these types, all homozygous for the two dominant markers. The number of ears in each pollination ranged from one to 15. The depth of aleurone color and pericarp translucency varied considerably, making the detection of mosaic areas more difficult in some families than in others. In doubtful cases the pericarp was removed and the mosaic area examined under a drop of water with a magnification of 10 to 20×. Brittle areas were cut and stained. Very small areas of only a few cells were difficult to differentiate clearly as to normal or brittle and these are not included in the tabulation.

## FREQUENCIES OF MOSAIC AREAS

In 14,505 seeds examined, 723 unpaired red aleurone areas were found without any growth changes. In 32 cases the red areas were paired with an adjacent dark colored area as described and illustrated previously (JONES 1937, plate 1b). Both dominant markers were removed in 214 cases; in these the red aleurone area was underlaid with a sunken, translucent endosperm that could be differentiated by staining from the surrounding tissues over which the surface color had not been changed. Red brittle areas were also paired with dark normal areas in 11 cases. The ratios of unpaired to paired areas in these two categories are as follows:

| | Unpaired | Paired with dark | Ratio |
|---|---|---|---|
| Red only | 723 | 32 | 23:1 |
| Red and brittle | 214 | 11 | 19:1 |

These two ratios are sufficiently alike to indicate a loss and relocation of linked genes where the breaks occur at random, and losses are more frequent than relocations.

In addition to the 723 instances of red areas, there are nine cases where the red areas coincide with an area that is raised above the surface of the seed. These growth alterations stained more darkly with iodine, the alteration extending toward the center of the seed in a variable mass of tissue usually cone shaped with the apex inside the seed and the base at the surface bulging outward. The darker staining indicates a denser starch formation possibly due to an increased number of starch granules. The individual cells are not noticeably larger.

Mosaic areas with cells larger than normal occur in all varieties of maize. These result presumably from a doubling of the number of chromosomes. Giant cells, also described previously, have a greatly increased number of chromosomes. In these cases there is usually no alteration in growth relation-

ship. The large areas showing increased cell size are not raised above the surface of the surrounding normal areas and show no irregularity in development. The outgrowths found are not due to an increase in cell size.

The outgrowths are not otherwise abnormal and do not suggest any pathological condition that could be related to neoplasms in animal tissue. They do show a change in control of cellular growth and arrangement. Other kinds of alterations of similar origin are more irregular in growth.

### PAIRED GROWTH CHANGES

The outgrowths also occur as one part of a paired red-dark mosaic. In eight cases the red area is raised; in two cases the dark part is raised. When the red area is also brittle, a raised condition presumably could not show, since the

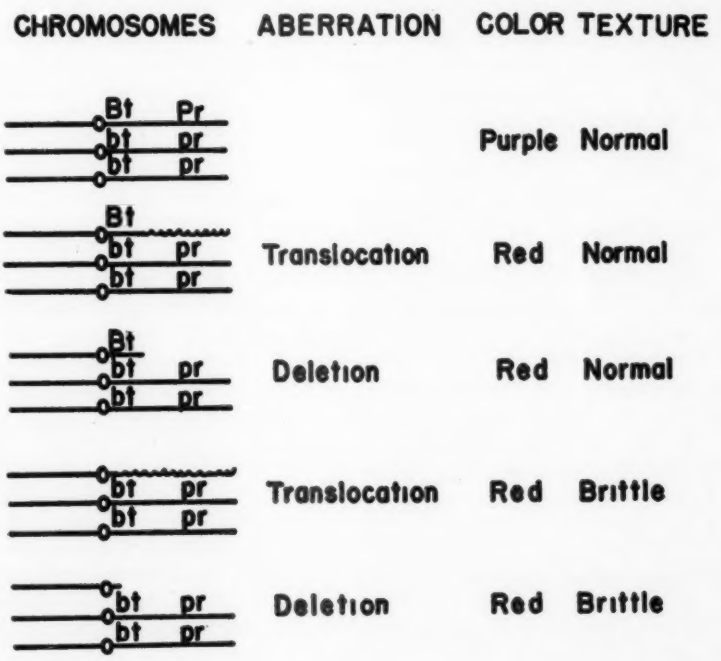


FIG. 2

brittle area is always shrunken below the surface of the seed at maturity. In 11 cases where red brittle areas are paired with dark colored areas, none of the dark areas is raised.

## INTERPRETATION OF GROWTH CHANGES

If the growth alterations are associated with relocation of the long arm of chromosome 5, any specific genes producing this effect would always be accumulated when the *Bt Pr* section was translocated. In 11 cases, where the removal included both *Bt* and *Pr* and, therefore, presumably, all of this arm to the right of *Bt*, there was no growth change in the dark area paired with the red, brittle area. In eight red-dark paired mosaics the red area was grown out. All of these facts show clearly that the growth alterations do not result from the accumulation of specific growth regulating genes by the translocation of parts of chromatids containing them.

It is seen, therefore, that in some cases the growth alteration accompanies the translocated piece. In other cases it remains with the chromatid from which the translocated piece has been removed. But in 32 cases, where red and dark areas are paired, no growth alteration took place in either area. How can all of these puzzling facts be given a consistent interpretation?

The breaking and relocation of chromosome parts in itself is not responsible for the result. The evidence is clear in showing that the outgrowth is not due to the uncovering of one or more recessive genes, or stated another way, to the removal of dominant regulatory genes located somewhere between *Pr* and *Bt*. Along with a total of 723 red areas that were not otherwise changed there were only nine red areas that were also raised, while at the same time there were 214 areas that were both red and brittle. On a linkage basis more red raised areas than red brittle areas would be expected from an uncovering of something between *Bt* and *Pr*.

The pairing is evidence that a reciprocal translocation has occurred. The growth change could result from bringing in some unknown regions from other chromosomes, thereby accumulating growth stimulating substances, but this would leave unaccounted for the two cases of red normal paired with dark raised where the accumulated region is known to be from the long arm of the number 5 chromosome.

The hypothesis that brings all the facts into line assumes that breaks must occur near certain places on the chromosomes and that the effect is the same whether the break occurs to the right or left of these growth activating regions.

The evidence does not prove that a relocation must always take place. However, the nine unpaired red spots that are also raised could be reciprocal translocations in which the paired part does not show. Either the accumulation of *Pr* genes has no visible effect, or the *Pr* part is not viable or does not grow in such a way as to reach the surface. All of these possibilities are known to occur in other material as previously described.

Also the evidence does not prove that any one specific locus must be brought into close contact with the activating region somewhere between *Bt* and *Pr*. Possibly there are many loci that can do this, otherwise the frequency of the growth changes would be even lower than it is. However the frequency is low and indicates very strongly that certain specific places only can produce the effect noted.

Other mosaic areas were found during this examination which are not included in table 1 or the summary in table 2. There were 15 raised areas that were not associated with any other alteration. They also stained dark when iodine was applied. On the hypothesis advanced, there are regions that can produce the same effect other than those on the long arm of chromosome 5. This would certainly not be surprising.

There were two brittle areas that were not red. They could be either mutations of *Bt* to *bt*, or internal deletions, or translocations not involving *Pr* or other unknown processes. Thirty-three seeds were variegated red or red and brittle as expected from repeated chromatid breakage and fusion as described by McCLINTOCK (1938). In 15 instances there were irregular depressions that

TABLE 2

*Summary and Ratio of Mosaic Areas in Table I.*

| DESCRIPTION AND NUMBER OF MOSAIC AREAS | | RATIO |
|---|---|---|
| Normal Growth | | |
| Red | Red-Dark Paired | |
| 723 | 32 | 23:1 |
| Red Brittle | Red Brittle-Dark, Paired | |
| 214 | 11 | 19:1 |
| Increased Growth | | |
| Red | Red Raised | |
| 723 | 9 | 80:1 |
| Red-Dark, Paired | Red Normal-Dark Raised Paired | |
| 32 | 2 | 16:1 |
| Red-Dark, Paired | Red Raised-Dark Normal, Paired | |
| 32 | 8 | 4:1 |
| Red Brittle-Dark Normal, Paired | Red Brittle-Dark Raised, Paired | |
| 11 | 0 | |

differed from the slightly shrunken brittle areas, and the tissues adjacent to these depressions did not give the characteristic brittle staining reaction. In many of these cases no aleurone color was present. These expressions appear to be failures of normal growth and food storage associated with chromosome irregularities as previously described (JONES 1937). These are more in the nature of a pathological change. Anything that would bring about a cell proliferation would be expected to result in a failure of food storage. Any areas of this kind would show as a depression when the seeds finally dried out.

## COLOR CHANGES NOT DUE TO GENE LOSSES

There were 52 light colored areas, the result of some change in the amount of pigment formed. The aleurone was clearly not red but simply a lighter shade of the normal bluish black color of the normal mature seeds. In 12 cases these light areas were paired with areas darker than normal. The ratio of nearly 4 to 1 is very high for unpaired and paired color changes. Although the parental

stocks were evenly colored red and purple, when these seeds were grown there was a segregation of light and dark cap in the second generation seeds. Evidently they were heterozygous for some aleurone color modifiers that did not show in the original stocks. In these segregating progenies there was no unusual variation in kernel size that might result from a heterozygous condition in growth regulating genes. The segregation of brittle and red was affected by one or more gametophyte factors. Some progenies had an excess of red brittle seeds, others a deficiency instead of the normal 25 percent. Gametophyte factors in the *Bt*, *Pr* region have been described by BURNHAM (1936).

## POSITION EFFECT

The evidence for position effect in plants has been reviewed by ROBERTS (1942) who has added results from induced reciprocal translocations in maize. Small changes were noted which could not be separated clearly from the effect of small deficiencies. Translocations in parts of the maize plant other than the endosperm are comparatively rare. In other genetic stocks second generation progenies segregating for white, virescent and glossy seedlings (these markers in different chromosomes) were treated with X-rays in the dormant seeds. No paired stripes were found in 2033 seedlings examined. In seeds heterozygous for three endosperm markers (*C*, *Pr*, *Su*, also in different chromosomes), 11 paired mosaic areas were found in 5625 untreated endosperms (JONES 1941). In maize embryos, chromatid relocation occurs at a low rate or is immediately cell lethal.

## GROWTH REGULATING GENES ON CHROMOSOME 5

In view of the possible position effect here recorded, it is interesting to examine the known genes in the region between *Bt* and *Pr*. Of the genes which have an effect upon growth there are: brevis, reduced endosperm-2, and dwarf-6. Two of these affect plant growth, one the endosperm. Other known genes in this region, in addition to the gametophyte factors noted above, are viriscent-3, vivipary-2, and glossy-6. Normally the plant and gametophyte factors have no visible relation to endosperm development and the reverse, but when chromosome fragments are moved to another location, it is possible that both endosperm and embryo growth regulators could produce cell stimulating substances. However, it is not necessary to assume that any known region is responsible for these cell alterations. It has been shown that somewhere on chromosome 5 between the loci *Bt* and *Pr* one or more points have a stimulating effect upon growth when brought into contact with other regions of the chromosomes which are normally kept separate.

## SUMMARY

Outgrowths of the surface of mature maize seeds are associated with breakage and relocation of chromosome parts.

Using gene markers of the long arm of chromosome 5 it is shown that somewhere between *Bt* and *Pr* growth activating regions exist which are stimu-

lated to increased growth persumably when brought into close contact with other regions of the chromosomes that are normally kept separate.

ACKNOWLEDGMENTS

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# OPPOSITIONAL ALLELES IN NATURAL POPULATIONS OF TRIFOLIUM REPENS[1]

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ALTHOUGH the multiple oppositional alleles which condition incompatibility have been described for a large number of species [review by STOUT (1938)], their occurrence in natural populations has been reported in only one previous study. EMERSON (1939) found 34 different alleles in a natural population of *Oenothera organensis* estimated at about 500 plants. He concluded, however, that the sample which was analyzed was too small to indicate the relative frequencies of different alleles in the entire native population. Extensive tests for the number of alleles were made similarly in three other species but in none was a natural population used. In Nicotiana EAST and YARNELL (1929) found 15 different alleles among the 32 which were tested. With red clover, WILLIAMS (1941) tested two series of plants, in which he found different alleles as follows: 37 out of 40, and 41 out of 48. In a test of two series of unrelated white clover plants (ATWOOD 1942b), 25 out of 26 and 34 out of 41, respectively, of the alleles proved to be different. With all four species the test for allelism consisted of making all possible combinations (diallel crosses) between plants which had one *S* factor in common. Incompatible matings indicated that the two unknown alleles being tested were identical, whereas compatible crosses indicated they were different.

Because white clover plants can be easily crossed in the greenhouse with decisive results and because natural populations are more readily available in white clover than in some other species studied for oppositional alleles, the frequency of these factors was studied in two representative white clover populations of different sizes.

## MATERIALS AND METHODS

The plants were collected in two pasture areas in central Pennsylvania, where white clover occurs naturally.[3] Both areas have been grazed almost continuously during every summer for as long as local records show, and both usually have had abundant moisture. In situations such as this, white clover is known to have become established at the time of the early settlement and probably to have persisted ever since.

Since abundant seed was found in nearly all heads examined in these fields,

[1] Contribution No. 62, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. DEPARTMENT OF AGRICULTURE, STATE COLLEGE, Pennsylvania, in cooperation with the northeastern states.

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[3] The kind assistance of DOCTOR F. V. GRAU, THE PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION, in locating and collecting these plants is gratefully acknowledged.

and since all the collected plants proved practically self-incompatible when tested subsequently in the greenhouse, it is reasonable to conclude that the seed observed in the field had resulted primarily from cross-pollination by the few bees seen visiting the white clover flowers. No honey bees were seen in these fields at the time of collection in 1941, and none were known to have been kept within normal flying range. It is probable that every year sufficient seed was produced to provide for the continuous survival of the species.

The first series, consisting of the smaller population of plants, was obtained from a single isolated pasture on Pennsylvania route 545 about half way between Pine Grove Mills and McAlevys Fort. For several miles in every direction this cleared pasture is surrounded by forest-covered mountains, where white clover is ordinarily not found. About two miles south were a few cleared fields, where cultivated crops apparently had been grown for some time, but no white clover was found there. In the pastured field, the clover was confined principally to a narrow strip along a creek and to two adjoining areas, one on the slope from the road and the other along an old fence row. Except for these areas the field was covered with weeds and ungrazed grasses. There was no evidence of plowing in recent years, and all vegetation suggested that the pasture was very old. The stand of white clover was very dense on the approximately acre-sized area where clover was confined. It is estimated that the total population was not more than a few thousand plants. Sixty-two small sod plugs were collected at random from this population. The plugs were dug at least 15 feet apart in order to minimize the chances of any two being vegetative increases of a single original plant. After the plants had grown for three weeks in the greenhouse, one stolon from each plug was transplanted to a new pot. From the 55 plants that flowered in the greenhouse, 49 were selected as parents of the $F_1$ plants to be used in the diallel crosses.

The second and larger population was obtained from a series of bottomland pastures adjoining Spruce Creek between Colerain State Park and the junction of Pennsylvania routes 45 and 45c. The area on which white clover was found was probably less than 100 acres, but was irregularly shaped. The air-line distance from one end of the pasture area to the other was about five miles, but the width varied from about one-half mile to only a few rods. The total number of white clover plants was estimated at a few hundred thousand. This population was isolated from other white clover areas by Tussey Mountain on the east side, by the "Barrens" woodland on the west, by the State Park at the south end, and by cultivated farm land at the north. The white clover pastures were not quite continuous, being interrupted by a few cultivated or ungrazed fields and by a few places where the creek flowed very close to the road. Since the pasture area was so long, it is improbable that bees regularly carried pollen over the entire area on a single flight. The area was so nearly continuous, however, that the clover population may be assumed to be freely interbreeding, at least by steps. The population was sampled by collecting two plugs at each of 50 locations, with no two locations being closer than 100 yards, and the average distance apart being over 200 yards. From each plug a single stolon was isolated

and vegetatively propagated. From each pair of plants obtained at the same location the one which grew and flowered better in the greenhouse was selected as parent of the $F_1$. In this way $F_1$ progenies were obtained from 49 of the 50 locations.

The parent common to all 98 $F_1$ progenies was homozygous for oppositional alleles and was the same plant used in the previous study (ATWOOD 1942b). Three to five $F_1$ plants from each of the 98 progenies were grown as spaced individuals in the field in 1942, and the most vigorous and most profusely flowering plant in each progeny was selected for use in the diallel crosses.

The method of emasculation and pollination in the greenhouse has been described (ATWOOD 1941).

## EXPERIMENTAL RESULTS

Each of the 49 selected parents in each series was compatible with the plant homozygous for oppositional alleles ($S_1S_1$). Crosses were made in both directions whenever flowers were available, but in most cases sufficient seed to obtain the desired $F_1$ progeny was secured from a single head with ten flowers. In the first series of plants, 47 were crossed as female with $S_1S_1$, and the seed set per head ranged from 15 to 57 with an average of 37.0. In the second series, all 49 plants were used as female, and the seed set ranged from nine to 53 with an average of 35.7. The difference between series was not significant. When 85 of the 98 selected plants were crossed as males with $S_1S_1$, the average seed set per head was 31.6.

The reflexing of the pedicels (ATWOOD 1940) within 48 to 72 hours after pollination proved a reliable criterion of the seed set to be expected. Whenever only part of the flowers were reflexed, the cross was repeated. In every such case, good seed set was obtained in the second trial, indicating that the poor reflection and the consequent lower seed sets probably resulted from mechanical damage during emasculation and pollination rather than from reduced compatibility. Consequently only the higher or highest seed set from any single compatible cross was used in computing averages.

The extra flowers, available on 93 of the 98 selected plants, were used to test for self-compatibility. With 91 of these plants, a total of 293 heads, each consisting of ten flowers, yielded a maximum of three seeds per head and an average of 0.16 following self-pollination. With 48 of these same plants, 183 entire heads that were manipulated to effect selfing yielded a maximum of six and an average of 0.52 seeds per head. It is evident that all these plants were practically self-incompatible. Each of the other two plants that were selfed produced an average of 7.0 seeds per head, when three and six heads, respectively, were selfed using ten flowers per head. Following manipulation, one of the plants yielded 32 and 39 seeds, respectively, in two heads, and the other produced seven seeds in four heads. Apparently these two exceptional plants were pseudo-self-compatible to a slight degree, but it has been shown previously (ATWOOD 1942c) that this character does not influence cross-incompatibility.

The parent homozygous for incompatibility alleles ($S_1S_1$) was also homo-

zygous for a dominant white marking on the leaf blade, a character which none of the 98 selected parents possessed. All $F_1$ plants growing in the field showed this white marking, indicating that they were legitimate progeny. Since it is essential in a test of this type that all plants to be diallely crossed have one allele in common, a further test was made by backcrossing each selected $F_1$ plant to its *S1S1* parent using the latter as male. Every cross proved incompatible as expected, indicating that all $F_1$ plants bore the allele *S1*. In the first series 59 heads on the 49 plants were crossed in this way, and the average seed set was 0.27 with a maximum of two per ten flowers. Likewise in the second series, 52 heads yielded an average seed set of 0.31 and a maximum of three.

When the 49 $F_1$ plants of the first series were diallely intercrossed, 36 of the 49 unknown genes, or 73.5 percent, were found to be different. As summarized in table 1, each of 27 genes were found in only a single $F_1$ plant, each of six

TABLE 1

*Frequency of different alleles in two natural populations of white clover.*

| POPULATION NUMBER | NO. OF DIFFERENT ALLELES EACH OF WHICH OCCURRED IN THE FOLLOWING NUMBER OF $F_1$ PLANTS | | | | TOTAL ALLELES TESTED | TOTAL DIFFERENT ALLELES | PERCENT DIFFERENT ALLELES |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | | |
| 1st series (smaller) | 27 | 6 | 2 | 1 | 49 | 36 | 73 |
| 2nd series (larger) | 30 | 8 | 1 | 0 | 49 | 39 | 80 |

genes occurred in two plants, each of two genes in three plants, and one gene in four plants. With this frequency of alleles, only 18 crosses (1.53 percent) were incompatible out of the 1176 possible combinations between the 49 $F_1$ plants. The final criterion of compatibility was the number of seeds obtained per ten flowers. In no case was there any question in distinguishing between compatible and incompatible crosses in this regard. Among the compatible crosses, eight percent were made reciprocally in order to check on results that appeared questionable in regard to pedicel reflection. Omitting duplicates but including reciprocals, the average seed set per ten flowers was 37.3 for the 1269 heads. In contrast, the average seed set per ten flowers for the 54 incompatible crosses, including duplicates, was only 0.72. The ranges in seed set for the compatible and incompatible crosses did not overlap.

With the second series of plants, 39 of the 49 alleles that were tested, or 79.6 percent, proved to be different. In this case each of 30 alleles occurred in only one $F_1$ plant, each of eight occurred in two, and one allele was found in three plants. This frequency of different alleles was somewhat higher than was found in the first series, and it resulted in a lower percentage of incompatible crosses. Here only 11 (0.94 percent) of the 1176 possible matings were incompatible. In this series also there was obtained a very striking difference between compatible and incompatible crosses in average seed set. The 1377 heads used

in the former, including 17 percent reciprocals, yielded an average of 38.1 seeds per ten flowers, whereas the 29 heads used in incompatible matings, including repeats, averaged only 0.81 seeds per ten flowers. With this series also the ranges did not overlap.

Since all plants of the first series were collected within the normal range for a single flight of a bee, no attempt was made to keep a record of the position of the sampled plants in the pasture. In the second series, however, a record was kept of their relative position, since the plugs were collected in consecutive order from one end of the long pasture area to the other, and since the area covered a much greater distance than the normal flight of a bee. If these points of collection are numbered continuously from one end to the other, the identical alleles were found in the following positions: 5-6-24, 8-12, 9-41, 11-38, 13–35, 16–30, 23–45, 31–44, and 36–40. The number of positions apart for the different pairs of identical alleles ranged from one to 32, with an average of 16. There is some evidence, therefore, that in this larger population there was a certain degree of local inbreeding within sub-populations, as postulated by WRIGHT (1939). A much more complete sampling would be necessary to prove this point conclusively.

It has been shown (ATWOOD 1940) that the average seed set per ten flowers is conditioned by the number of ovules per ovary and is significantly correlated with seeds set per head in the field. In order to complete all the diallel crosses in these studies, more crosses per female and more combinations of different males on a single female were obtained than in any previous work with white clover. Consequently, the variation found in these 98 $F_1$ plants should give a better sample and a more valid test of significance than was obtained previously. When the 98 plants were averaged as females, the range in average seed set per plant was very large, and the differences between plants were highly significant in both series (table 2). When the same plants were averaged as males, the ranges were much smaller in each of the series, but the differences

TABLE 2

*Summary of variation in seed setting ability for different $F_1$ plants and the analyses of these differences.*

| POPULATION NUMBER | | AVERAGE NUMBER OF COMPATIBLE MATINGS PER PLANT | RANGE IN AVERAGE SEED SET PER PLANT | ANALYSIS OF VARIANCE | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | BETWEEN PLANTS | | WITHIN PLANTS | | F |
| | | | | D.F. | MEAN SQUARE | D.F. | MEAN SQARE | |
| 1st series | ♀ | 25.9 | 18.4–47.8 | 48 | 1276.4 | 1220 | 50.4 | 25.34** |
| | ♂ | 26.4 | 25.8–41.4 | 47 | 246.1 | 1221 | 91.0 | 2.70** |
| 2nd series | ♀ | 28.2 | 19.9–50.8 | 48 | 1448.1 | 1335 | 66.2 | 22.19** |
| | ♂ | 28.2 | 26.2–41.4 | 48 | 211.8 | 1335 | 110.6 | 1.91** |

** Exceeds F for P = .01

were still highly significant. In the first series, however, 44 of the 49 plants were within the range of the least significant difference at the one percent point. The remaining five plants either were noted as poor pollinators or were used in too few crosses to be sampled adequately. Likewise in the second series 47 of the 49 plants were within the same range. One of the other two plants was noted as a poor pollen producer at the time of the crossing, and the second was inadequately sampled. It is concluded that these plants differ significantly in their seed setting ability as females, but as males the differences between the same plants are questionable, especially among those plants with no obvious morphological defect.

Five of the plants were found to have such low female fertility that in a few cases there might have been difficulty in deciding whether a particular cross was compatible or incompatible if it had not been possible to check all such crosses reciprocally. With these five plants, not only was the mean seed set low, but the variation between heads was greater in proportion to the mean than in the case of most other plants. Morphological abnormalities rather than changes in compatibility seemed to be responsible for most of this variation.

In all previous compatibility studies with white clover, the male parent has been shown to have no significant effect on the seed set obtained from a compatible cross, except, of course, those males with anthers so hard that pollen is removed only with great difficulty (ATWOOD 1942a). Except for a single plant, similar results were obtained with these series. One of the $F_1$ plants in the first series was found to be highly male sterile, the first such case described for white clover. When the diallel crosses were made during the winter, the pollen of this plant was used in crossing 13 heads, and it was not observed to be abnormal in any way. The reflection of the pedicels was noted as being slow with two of these heads, but otherwise there was no indication that normal seed was not being formed. When these heads were threshed, however, they bore a maximum of four and an average of only 1.23 seeds per head. On the other hand, this plant was completely female fertile. When it was crossed with the 48 other $F_1$ plants, the seeds per head ranged from 31 to 50 and averaged 42.6. Included among these 48 crosses were the reciprocals of the 13 which had set little or no seed using the plant as male. When cuttings of this plant were brought into flower again, microscopical examination of the pollen showed no unusual characters either in size or in stainability with aceto-carmine or with iodine and potassium iodide solution. A preliminary cytological examination of the microsporocytes likewise has shown no irregularities. A further study of this character is now being made.

## DISCUSSION

When a large proportion of the alleles, found in the two series of unrelated plants tested previously (ATWOOD 1942b), were observed to be different (96 and 83 percent, respectively), it was reasoned that the diversity had resulted from sampling a very large population. In the relatively smaller natural populations described here, the percentages of different alleles (73 and 80 percent,

respectively), proved surprisingly similar to those of the unrelated plants, but, without more replication, the significance of the differences cannot be adequately tested. The fact that both natural populations are lower, however, suggests that population size within the limits tested may be a contributing factor to the proportion of different alleles. The theoretical aspects of this problem have been discussed by WRIGHT (1939).

A large number of oppositional alleles have been found both in white clover and in other species, whereas multiple alleles have been only infrequently described for most other characters and never in such large numbers. It might be speculated either that the oppositional alleles are of quite a different type than most other genes in having greater selective advantage, or that they are similar to others with the differences more easily demonstrated because of the nature of the test. Perhaps if non-heritable variation could be eliminated as easily with other genes and if a specific test could be developed for relatively slight differences, it would be possible to demonstrate extensive series of multiple alleles for most loci. In any case the large diversity in oppositional alleles suggests that this source of evolutionary variation may be even more extensive than previous estimates have allowed.

From a breeding standpoint it is apparent as a result of this study that unrelated plants may generally be assumed to be cross-compatible. It can also be concluded that when plants are combined in a strain, a slight degree of relationship will not hamper relatively free intercrossing. When 73 percent of the alleles were different among the 49 plants tested from the smaller population, 98.47 percent of the diallel crosses were compatible. Consequently, under field conditions it is highly improbable that incompatibility would be a deterrent to seed set, since some compatible pollen would likely reach almost every flower as a result of promiscuous pollination by bees.

## SUMMARY

In each of two natural populations of white clover, estimated at a few thousand and a few hundred thousand individuals, respectively, 49 plants were sampled for a study of the frequency of oppositional alleles. All 98 plants proved self-incompatible, and all were cross-compatible with a plant homozygous for oppositional alleles ($S_1S_1$). One plant was selected in each $F_1$ progeny, and diallel crosses were made between the 49 $F_1$ plants within each series. Incompatible matings among the diallel crosses indicated that the two unknown alleles being tested were identical.

In the first series, 36 (73 percent) of the 49 alleles were different, resulting in 1.53 percent of the matings being incompatible. In the second series, 39 (80 percent) were different, resulting in only 0.94 percent incompatible crosses.

Highly significant differences between plants within each series were found in respect to number of seeds per ten flowers following compatible crosses.

The first case is described for white clover of a plant being highly male sterile due to other than morphological causes.

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# THE EFFECT OF X-RAYS UPON THE ACTION OF A SPECIFIC GENE IN DROSOPHILA MELANOGASTER

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A NUMBER of workers in the past few years have used X-ray treatments to induce phenocopies. FRIESEN (1935, 1936) appears to have been the first to do this. HASKINS and ENZMANN (1937) and ENZMANN and HASKINS (1938a, 1938b), LOBASHOV and SOLODOVNIKOV (1939), GRATZIANSKY (1939), WADDINGTON (1942a, 1942b), BLANC and BRAUN (1942), and BLANC and VILLEE (1942) have all used this technique. Most of these workers, following FRIESEN, used moderate to heavy doses (2000–7000 r units) and treated individuals of advanced larval, prepupal or pupal stages, since these had been shown by MAVOR (1927) and WOSKRESSENSKY (1928) to be especially sensitive stages. FRIESEN (1936) reports obtaining, in general, lower percentages of phenocopies ("Röntgenmorphosen") from the treatment of young larvae, 24 to 48 hours after egg-laying, than of older stages. GRATZIANSKY (1939) found that the highest frequency of "Notch" phenocopies was obtained by treating 66-to 96-hour old larvae. BLANC and BRAUN (1942) and BLANC and VILLEE (1942) confined their treatments to prepupal and pupal stages. WADDINGTON (1942b) treated individuals of larval stages as well as those further advanced, but reported that only normal individuals hatched from larvae less than 48 hours old at the time of treatment. Only HASKINS and ENZMANN (1937) and ENZMANN and HASKINS (1938a, b), using low doses of 40 to 312 r units, have obtained what were probably phenocopies by treating eggs or very young larvae less than 24 hours old. They reported finding melanotic tumors, retention of the anterior larval spiracles in the imago, and eyes that were bilobed or had irregular ommatidia, sunken areas, scars or "tumors," the latter usually white in color and of a palp-like structure. These abnormalities they interpreted as the result of chromosome fragmentation, producing deficiencies such as PATTERSON (1929, 1930) found in his analyses of mosaic individuals produced by the X-ray treatment of eggs and young larvae; but there is no evidence that they were such rather than phenocopies, since these workers did not use the techniques of PATTERSON for detecting deficiencies.

In none of the work cited above was the action of X-rays shown to be linked with any specific gene-controlled process, as in the instance to be reported in this paper. More nearly parallel phenomena have been described by DANEEL and LUBNOW (1936) and LUBNOW (1939), and by SMITH (1942). DANEEL and LUBNOW, and LUBNOW, following up an early discovery made by HANCE and MURPHY (1926) and HANCE (1927) that X-rays will inactivate pigment formation in the hair of mice, have been able to show that the precursor reaction (reaction I) in the formation of rabbit melanins is inactivated by X-rays, a reaction further known to be under the control of the Dutch spotting genes.

SMITH has discovered an "X-ray susceptible factor" in *Triticum monococcum*. Plants with this recessive gene in the homozygous condition show a very marked sensitivity to X-ray doses of 4000 to 20,000 r units, resulting in "pronounced flecking on the leaves, slower growth, and death from smaller doses than are required to kill normal seeds." In the case to be reported here, X-rays of relatively low dosage (1000 r), applied at an early stage of embryonic development to Drosophila individuals of a particular genetic stock, produced in nearly every treated fly an inactivation of a specific gene-controlled process, that bringing about the suppression of a semi-dominant eye deformity carried by the stock.

## EXPERIMENTAL DATA

In the summer of 1941, eggs of *Drosophila melanogaster*, from a brown scarlet (*bw; st*) stock furnished by Dr. H. J. MULLER, were X-rayed at the age of $10 \pm 1$ hours (26°C) with a dosage of 1000 r units (85 kv, 5 ma, 15 cm distance, filters 1 mm Al+cardboard; ca. 250 r per min.) (brown, eye color, chromosome 2, 104.5; scarlet, eye color, chromosome 3, 44.0). The larvae hatched in normal numbers and appeared vigorous throughout all larval instars. They also pupated normally, but there was a high mortality at hatching. Those flies which succeeded in emerging as adults were weak, with legs frequently crippled, and most of them became stuck in the food within a short time and died. Nearly all of these flies had black growths, probably tumors, inside their abdomens. Males as a rule were too feeble to mate. Of the 221 flies examined, 90 out of 94 ♂♂ and 118 out of 127 ♀♀, amounting to 94.1 percent of all individuals, were observed to have a peculiar growth located approximately in the center of either eye, or more usually of both eyes. This growth varied in appearance from a mere protrusion of the eye in the central area to an eruption of the underlying tissues, so as to form a slender twisted structure often bearing bristles and sometimes appearing to be segmented. To this characteristic the designation of erupt (symbol—*er*) was given. It resembles the mutants ophthalmopedia (GORDON 1936), kidney$^2$ and kidney$^3$ (GOTTSCHEWSKI and MA 1937), and the "kidney" mutant of VALADARES (1937). It is not, however, an allele of any of these (see below).

Ten hour old eggs from an *al b c sp*$^2$ stock were treated simultaneously with those from the *bw; st* stock. (*al*, aristaless, chromosome 2, 0.0; *b*, black, 2, 48.5; *c*, curved, 2, 75.5; *sp*$^2$, speck$^2$, 2, 107.0.) The emerging flies were feeble and frequently crippled and tumorous, as in the case of the *bw; st* treated flies, but in not a single individual was the erupt characteristic to be observed.

The treatment of eggs of the *bw; st* stock was repeated in the following summer of 1942, and the existence of the phenomenon was confirmed. Eggs $10 \pm 1$ hours old (24.5°C) were given a dose of 1000 r units. The results for both years are recorded in table 1. In view of the fact that in counting eyes rather than individual flies the proportion classed as normal would be somewhat higher, it is evident that there is no appreciable difference between the results of the two years.

TABLE 1

*Effect of treating ten-hour old bw; st embryos with 1000 r units of X-rays.*

| | UNIT OF CLASS. | EYES NORMAL | EYES ERUPT | |
|---|---|---|---|---|
| | | | WEAK | STRONG |
| 1941 | fly | 13 | 208 | |
| 1942 | eye (½ fly) | 12 | 18 | 148 |
| untreated control (1942) | eye (½ fly) | 467 | 30 | 3 |

Forty-nine erupt ♀♀ and 30 erupt ♂♂ of the treated *bw; st* stock were mated with untreated *al b c sp*$^2$ ♂♂ or ♀♀, and ten of the females produced small progenies, the remainder being totally sterile. All offspring, 273 in number, were completely normal-eyed (with the exception of one ♂ that had an eye resembling the mutant Lobe, presumably the effect of a mutation induced by the X-ray treatment). It thus originally appeared as though the X-ray treatment had directly induced a specific somatic effect in virtually all the treated flies of a particular stock. This, however, turned out not to be the correct explanation, for, as was seen from the next crosses made, a mutant responsible for the erupt character was already present in the *bw; st* stock. This was further confirmed by the occasional appearance of flies with weak erupt or even strong erupt eyes in the untreated stock culture, as may be seen in the control count made in 1942 given in table 1.

Individual males from the outcross progeny were next backcrossed to several *bw; st* females from the original stock. The four expected types of eye color appeared in the following proportions: red (*bw*$^+$ *st*$^+$) 977; brown (*bw st*$^+$) 858; scarlet (*bw*$^+$ *st*) 771; light orange (*bw st*) 618. The erupt eye character also made a reappearance in this generation, showing up in 32 wild type, 0 brown, 129 scarlet, and 6 brown scarlet flies. In examining the flies of this generation, it was noticed for the first time that many of them had slight disarrangements of the ommatidia, usually in the center of the eye where the extrusion takes place in erupt individuals. These slight disarrangements were found to vary in frequency within the four classes in the same manner as erupt: wild type, 107;

TABLE 2

*Progeny of backcross of F*$_1$ *bw; st/al b c sp*$^2$ ♂♂ *(from treated bw; st* ♀♀*) to bw; st* ♀♀.

| | + | % | BROWN | % | SCARLET | % | BROWN SCARLET | % |
|---|---|---|---|---|---|---|---|---|
| non-erupt | 838 | | 839 | | 407 | | 559 | |
| weak erupt | 107 | 11.0 | 19 | 2.2 | 234 | 30.3 | 53 | 8.6 |
| strong erupt | 32 | 3.3 | 0 | 0.0 | 130 | 16.9 | 6 | 1.0 |
| Totals | 977 | | 858 | | 771 | | 618 | |

brown, 19; scarlet, 234; brown scarlet, 53. Some of the disarrangements, which often involved only two or three facets, may have been due to other factors, but it seems safe to regard the majority of them as slight expressions of erupt. In table 2 the data for the backcross are summarized. This summation conceals the fact that there was very great heterogeneity in the frequency of total erupt and of strong erupt in individual cultures. The analysis of the factors producing this and the related marked differences in the dominance of erupt in various crosses are being further investigated. In the first of the four types of eye colors, both chromosomes 2 and 3 from the *bw; st* stock are carried in the heterozygous condition. In the brown-eyed flies, both second chromosomes are from the *bw; st* stock but only one of the third chromosomes. In the scarlet-eyed flies, both third chromosomes come from the *bw; st* stock, but chromosome 2 is heterozygous. In the double recessive type, both pairs of major autosomes come from the *bw; st* stock. The difference in the frequency of the erupt character in these four classes may therefore be interpreted as the result of the independent segregation of two pairs of factors (or factor complexes): one in chromosome 3 responsible for the erupt character, and one in chromosome 2 acting as a suppressor of erupt (*Su-er*). The erupt factor is more effective when homozygous than when heterozygous (compare the incidence in the scarlet and brown scarlet classes with that in the normal and brown classes, respectively). Yet erupt is not completely recessive, for erupt eyes may appear in flies heterozygous for *er*—namely, in the wild type flies. The suppressor is evidently far more effective when homozygous than when heterozygous (compare the incidence of erupt in the brown and brown scarlet classes with that in the wild type and scarlet classes, respectively). That the suppressor, like erupt, is also partially dominant is shown by the results of another cross, in which *b Su-er*$^{+}$ *sp*$^{2}$*; st er* ♀ ♀ were crossed with *bw Su-er; st er* ♂ ♂. In a total of 677 individuals in nine progenies, there were 86 normal eyed, 425 weak erupt, and 166 strong erupt. A control count of 119 individuals from the *b Su-er*$^{+}$ *sp*$^{2}$*; st er* stock yielded 1 normal eyed, 7 weak erupt, 111 strong erupt. From the comparison it is quite clear that the *Su-er* gene from the original *bw Su-er; st er* stock is partially dominant, changing the median manifestation of erupt from strong to weak.

Further evidence that the treated *bw; st* stock contains a factor in the third chromosome responsible for the erupt effect was obtained by selecting and crossing together, from the progenies of the previous group of crosses, ♂ ♂ and ♀ ♀ that were scarlet as well as strongly erupt. These would presumably carry erupt, like *st*, in the homozygous condition, but the suppressor in chromosome 2 in the heterozygous state. From the next generation, flies that were scarlet and strong erupt, and incidentally black brown as well, were selected and used as the beginning of a line that lacked the suppressor from the *bw; st* stock, and carried instead the gene (or genes) from the *al b c sp*$^{2}$ stock permitting strong manifestation of erupt. The frequency of strong erupt in this new stock is 88 percent. Flies from the new stock were then crossed to *Pm/Cy; D*$^{3}$ *H/Sb-C* flies, and $F_1$ ♂ ♂, *Cy; D*$^{3}$ *H*, were back-crossed to *b bw; st er* ♀ ♀. (*Pm*,

Plum, eye color, inseparable from inversion of 2LR; *Cy*, Curly, wings upcurled, inseparable from inversions 2L, 2R; $D^3$, Dichaete[3], wings outstretched, chromosome 3, 40.4; *H*, Hairless, absence of certain bristles, chromosome 3, 69.5; *Sb-C*, Stubble, short thick bristles, chromosome 3, 58.2, associated with inversion 3R.) The results are given in table 3.

In this cross erupt is completely recessive to its allele in the $D^3$ *H* chromosome whenever this is accompanied by the second chromosome carrying *Cy*, and shows only a slight penetrance whenever the *Cy* chromosome is replaced by that from the new selected stock. However, when erupt is homozygous, the replacement of the *Cy* chromosome 2 by that from the new stock results in a marked increase in the frequency of strong erupt. The *Cy* chromosome 2

TABLE 3

*Progeny of backcross of $F_1$ Cy/b bw; $D^3$ H/st er ♂♂ to b bw; st er ♀♀*

| | NON-ERUPT | WEAK ERUPT | STRONG ERUPT | PERCENTAGE STRONG ERUPT | TOTAL |
|---|---|---|---|---|---|
| *Cy $D^3$ H* | 98 | 0 | 0 | 0.0 | 98 |
| *Cy st* | 24 | 28 | 39 | 42.9 | 91 |
| *b bw $D^3$ H* | 81 | 2 | 3 | 3.5 | 86 |
| *b bw st* | 6 | 10 | 46 | 74.2 | 62 |
| Total | 209 | 40 | 88 | | 337 |

evidently carries a suppressor of erupt like that present in the original *bw; st* stock.

The attempt was also made to locate more exactly the genes responsible for erupt and for the suppressor of erupt in the third and second chromosomes respectively. This task was rendered difficult by the partial dominance of both erupt and its suppressor. Flies from a stock carrying the third chromosome combination of recessive mutants known as "rucuca" (*ru h th st cu sr $e^s$ ca*) and also *er* were crossed with wild type flies, and $F_1$ ♀♀ were backcrossed. (*ru*, roughoid, 0.0; *h*, hairy, 26.5; *th*, thread, 43.2; *cu*, curled, 50.0; *sr*, stripe, 62.0; $e^s$, sooty, 70.7; *ca*, claret, 100.7.) Three hundred and twenty-eight offspring, from three cultures, were classified without obtaining data capable of giving a clear-cut analysis, mainly owing to three reasons: (1) there were 93 flies with a slight manifestation of erupt, and without extensive testing it could not be determined whether these were heterozygous or homozygous; (2) in flies manifesting roughoid, the classification of erupt is uncertain; (3) claret seems to act as, or to be associated with, a suppressor of erupt. No effort was made to secure a large progeny from this cross when it was realized that the analysis and interpretation of the data would be unreliable. New stocks combining erupt with third chromosome recessives are being made up for an adequate testing of the location of erupt. However, the 86 flies from the above progenies that manifested erupt strongly may be used for a tentative analysis. Among

these 86 there were nine crossovers between erupt and $e^s$ (10.5 percent), 27 between erupt and *cu*, 31 between erupt and *sr*, and 43 between erupt and *ca*. This is enough to show that erupt behaves as if determined by a single gene, and that this gene is closer to $e^s$ than to any other of the marker genes. Whether it is to the right or to the left of $e^s$ must remain uncertain for the present.

When *er* was found to be closely linked to $e^s$, it seemed probable that it might be an allele of kidney similar to kidney[2] and kidney[3], descriptions of which tally very closely with the phenotype and variability of erupt. Accordingly, a cross was made to test this possibility. Females, *b Su-er*$^+$ *bw; st er*, strongly erupt in phenotype, were mated with *ss bxd k* $e^s/Xa$ ♂♂ (*ss*, spineless, 3, 58.5; *bxd*, bithoraxoid, 3, 58.8±; *k*, kidney, 3, 64.0±; *Xa*, Xasta, associated with a translocation between chromosomes 2 and 3). The progeny from this cross are classified in table 4. Since the penetrance and degree of manifes-

TABLE 4

*Test for allelism between erupt and kidney. Progeny of cross b Su-er*$^+$ *bw; st er* ♀♀ × *ss bxd k* $e^s/Xa$♂♂.

| | *Xa/er* | *k/er* | |
|---|---|---|---|
| Normal eyed | 6 | 50 | |
| Weak erupt | 56 | 18 | |
| Strong erupt | 11 | 1 | |
| Totals | 73 | 69 | 142 |

tation of erupt in heterozygotes *er/k* are considerably less than in the heterozygotes *er/Xa*, it seems clear that *er* and *k* are not alleles and that the presence of strong and weak erupt flies in the progeny of this cross is due to partial dominance on the part of erupt.

To determine the location of *Su-er* in chromosome 2, *b Su-er*$^+$ *sp*$^2$*; st er* ♀♀ were crossed in pair cultures with *bw Su-er; st er* ♂♂, and the $F_1$ ♀♀ with normal or very weak erupt eyes were test-crossed to *b Su-er*$^+$ *sp*$^2$*; st er* ♂♂. In classifying the test-cross progenies it was assumed that a weak manifestation of erupt was always due to the presence of the suppressor in heterozygous condition, and that strong erupt eyes are found only in flies homozygous for the allele of suppressor-erupt (*Su-er*$^+$). That this assumption involves no very great error is clear from the frequency of strong erupt in the eight progenies. Where, with equal viability for erupt and non-erupt, 50 percent of flies with strong erupt eyes are to be expected, the frequencies obtained ranged from 38.0 to 50.4 percent, and the mean for the manifestation of strong erupt in all progenies combined was 45.9 percent. The test-cross data are summarized in table 5. Crossing over between *b* and *Su-er* ranged from 6.9 to 18.7 percent in the eight cultures, with a mean of 12.4 percent for all progenies combined. Although *sp* is too far away from both *b* and *Su-er* to serve as a very useful

marker, the presence of more flies in the complementary classes *b Su-er* and *sp; er* together than in the complementary classes *b Su-er sp* and *er* together, indicates that the two latter are more probably the double crossover classes. This would place *Su-er* to the right of *b* on the genetic map of chromosome 2, in the neighborhood of *cn*, cinnabar, 57.5. (Total detected crossing over between *b* and *sp* in these cultures amounted to 56.6 percent, compared with a standard map distance of 58.5. This is higher than is to be expected with so long an unmarked distance between *Su-er* and *sp*. It is therefore probable that *Su-er* is somewhat closer to *b* than the crossing over percentage obtained indicates.)

All the evidence at hand therefore indicates that a partially dominant gene

TABLE 5

*Test-cross for the location of suppressor-erupt in chromosome 2. Summary of eight progenies*

*from* $\frac{b \quad Su\text{-}er^{+} \quad + \quad sp^{2}}{+ \quad Su\text{-}er \quad (bw) \quad +}$; $\frac{st\ er}{st\ er}$ ♀♀ × *b Su-er*$^{+}$ *sp*$^{2}$*; st er* ♂♂.

| | |
|---|---|
| *b sp; er* | 418 |
| *Su-er* | 557 |
| *b Su-er* | 58 |
| *sp; er* | 70 |
| *b; er* | 356 |
| *Su-er sp* | 400 |
| *b Su-er sp* | 55 |
| *er* | 62 |
| Total | 1976 |

for erupt is present in the third chromosome of the original *bw; st* stock, and that its manifestation in that stock is suppressed through the action of a partially dominant gene, suppressor-erupt, in the second chromosome of that stock. This same suppressor, or one similar to it, is also present in the well-known *Cy* second chromosome.

## DISCUSSION

The action of the X-rays on the 10-hour old embryos of the *Su-er bw; st er* stock, as the original *bw; st* stock may now be designated, is therefore to be interpreted as an inhibition of the action of the suppressor of erupt. As to the mechanism by which this takes place, three possibilities are to be considered.

(1) The inhibition might be the result of induced gene mutation of the suppressor. Since the irradiation was applied to a multicellular embryo, yet proved effective in virtually all individuals, this would mean that a relatively low dose of X-rays produced mutation of a particular gene at an unprecedentedly high frequency. This hypothesis may be safely ruled out.

(2) The inhibition might be due to induced deficiencies that include the suppressor gene. This is the hypothesis held by ENZMANN and HASKINS (1938b) to account for the effects they found when treating eggs and very young larvae with low doses of X-rays. Like the previous hypothesis, this one seems highly improbable, since the frequency of deficiencies induced in a single chromosome by equivalent doses of X-rays administered to spermatozoa must be less than one percent. (BAUER, DEMEREC, and KAUFMANN (1938) give a figure of 3.63 percent for all types of chromosomal aberrations induced by 1000 r units in all chromosomes.) If the hypothesis is valid, a frequency of deficiency in somatic cells far higher than this would seem to be required. It remains possible that the suppressor gene produces a greatly weakened chromosome prone to break at a vastly increased rate. However, unless restricted to the particular tissue destined to form the center of the eye, deficiencies, unless very small, would be expected to produce other types of derangement in patterns of association like those found by MCCLINTOCK (1938) in her studies of ring chromosome deficiencies in maize. No such patterns were evident here.

(3) It seems considerably more probable that the X-rays do not directly affect the suppressor gene itself or the chromosome in which it is carried, but act upon some step in the development of the eye. This is the general assumption of most workers who have produced developmental effects and phenocopies by means of X-rays, heat, or other agents. This basis of interpretation is well supported by the work of LUBNOW (1939) on the histological results of treating the skin and hair of rabbits with X-rays. He has shown that in the treated areas, where the colored hair has been replaced by white hair, the matrix cells of the hairs differ from normal matrix cells in resembling ordinary epithelial cells morphologically and in lacking the specific cytoplasmic granules of a lipoid nature, termed by him the lipochondria, which are present in normal matrix cells. He has also shown that in the white-haired areas of Dutch spotted rabbits, the matrix cells also are of an ordinary epithelial type and lack lipochondria. In the white hairs of Himalayan rabbits, on the other hand, the matrix cells appear normal and possess the usual quantity of lipochondria. The effect of the Himalayan allele of the albino series has been interpreted as an intervention in the formation of an enzyme necessary for pigment formation. The Dutch spotting genes and the X-rays therefore appear to act on a still earlier stage of the reaction series, to intervene in an antecedent or precursor reaction (reaction I). The Himalayan gene is then concerned in the formation of the enzyme from the precursor. A direct action of the X-rays on the cytoplasm of the matrix cells and a dissolution of the lipochondria would seem to be a simpler and more probable mode of action than an indirect action through the genes and chromosomes. In the suppressor-erupt, erupt situation here studied, such an action might consist either of an inactivation (or decrease in activity) of the suppressor reaction system or of an increase in activity of the erupt reaction system, although it is questionable whether these can actually be separated.

One cannot help but be impressed by the extremely early age at which a

process, apparently concerned solely with a structure functional only in the imago, is yet capable of being affected. According to POULSON (1937), the frontal sac, from which the cephalic complex including the optic disk is formed, first appears quite late in embryonic development—17–18 hrs. at 22–23°C. From this it would seem certain that in the 10-hour old embryos irradiated in these experiments the frontal sac had not yet invaginated. Further experimentation must be made to determine whether the effect of the X-rays is general, later having a specific "after-effect" on the eye, or whether the effect is already localized in certain hypodermal cells determined as eye-forming. The latter seems somewhat more probable, in the light of the generally autonomous character of development in Drosophila, as well as because of certain results obtained by GEIGY (1931). GEIGY treated various stages of the embryonic period (½–24 hours) with ultraviolet rays and found that in the period from seven hours after egg-laying to hatching of the larva it is possible to induce abnormalities of wings, first legs, second legs, third legs, and sternites of the imago, in that sequence. Like the generalized crippling effects produced in the imago by the X-rays in the present experiments (the larvae remaining wholly unharmed), these results appear to show that independent imaginal anlagen exist in the young embryo.

If the suppressor reaction should prove to have an autonomous type of development, this would mean that the determination of parts of the imaginal disks takes place before the larva hatches from the egg and considerably earlier than any morphological determination is apparent. On the other hand, as STEINBERG (1941) and WADDINGTON (1942a) have pointed out, the determination of imaginal structures cannot in all cases be absolute, even late in the larval period. A certain degree of lability may be retained into the pupal stage itself. As WADDINGTON has emphasized, determination is a relative term, and the same mass of tissue that behaves as determined under one set of experimental conditions may appear still labile when the treatment is changed.

Another feature of interest in the present case is the homoeotic character of erupt, and its evolutionary relation to its suppressor and other modifiers. These will be considered at greater length in another paper. However, it may be pointed out at the present time that erupt resembles other homoeotic mutants in manifesting great variability in phenotype ("Expressivität") and in penetrance, and in showing bilateral symmetry only as a coincidence of the independent probabilities of manifestation on the right and left sides. The variability is to a considerable extent governed by genetic modifiers. These exist not only in the stock in which erupt was discovered in a suppressed condition but are present in a number of other stocks which do not carry erupt at all. The widespread existence of modifier systems tending to inhibit the effects of mutation of those genes which control key points in differentiation is to be expected. If, as GOLDSCHMIDT (1940, p. 329) has suggested, all imaginal disks have identical potencies in the embryo or early larva, the homoeotic mutants may be regarded as such key genes. The evolution of such a system may be conceived as comprising (1) the appearance of the original differentiating gene,

(2) the evolution of modifier systems governing its dominance, and (3) the evolution of modifier systems inhibiting the expression of mutations of the gene, even when in homozygous condition. In past discussions of this problem, attention has been chiefly focussed on the modifiers of dominance. It seems likely, from the study of the present case, that the inhibitory modifiers are equally widespread and of as great importance.

## SUMMARY

1000 r units of X-rays applied to 10-hour old embryos of a particular stock of *Drosophila melanogaster* resulted in the production of palp-like bristled protrusions from the center of the eye in approximately 95 percent of all individuals treated.

This effect has been shown to be due to the specific inactivation of a suppressor gene segregating independently of the homoeotic mutant responsible for the erupt effect.

Various stocks tested, including those lacking the mutant erupt, carry this or similar suppressors of erupt.

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# INHERITANCE IN NICOTIANA TABACUM. XVIII. MONOSOMIC ANALYSIS

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THE FIRST accounts of monosomics in *Nicotiana Tabacum* (CLAUSEN and GOODSPEED 1926a, b) reported establishment of two types and demonstrated that one of them, haplo-C (then called "corrugated"), involved the chromosome in which the basic color factor, *Wh*, is located. These results were the beginning of a program designed to establish as many monosomic types in *Tabacum* as possible and to take advantage of their favorable features for analytical studies of Mendelian characters. The investigations have now progressed to a successful isolation of 24 different monosomics which are believed to represent a complete set of the primary types.

## THE PRODUCTION AND MAINTENANCE OF MONOSOMIC STOCKS

Of the first two monosomics, haplo-F appeared as a spontaneous variant in a normal progeny and haplo-C was isolated as a derivative type from a cross between *Tabacum* and *sylvestris*. OLMO (1935) has described how monosomics may be isolated by design from crosses between *Tabacum* and *sylvestris* or *tomentosa* followed by recurrent backcrossing to *Tabacum*. Theoretically this method should lead to isolation of all possible monosomic types; in practice, however, the considerable heterozygosity set up in these crosses has been found to confuse the recognition and establishment of types. Nevertheless, a number of monosomics have been secured by this method, and recurrent backcrossing eventually does eliminate the troublesome segregation due to heterozygosity.

In more recent years, the asynaptic *Tabacum* type, pale-sterile, performs a similar service without the deterrent features of gene segregation, since pale-sterile has the same residual genetic constitution as the normal type. Pale-sterile has, on the average only 11 bivalents per microsporocyte, the rest of the chromosomes remaining unassociated. The progenies obtained from crosses of pale-sterile ♀ × normal ♂ consist of plants of marked diversity, as a consequence of random distribution of unassociated chromosomes. The variation is so considerable that it is difficult to find two plants exactly alike among the offspring. Aside from an occasional triploid, the offspring consists of unbalanced diploids. Relatively few, however, are simple monosomics or trisomics; most of them are more complex—double or triple monosomics, monosomic trisomic combinations, etc. These complex types, however, may readily be reduced to their elementary components by further crosses to normal.

The use of pale-sterile has another advantageous feature—namely, that in certain circumstances it permits isolation of specific monosomics by design. As an example, a cross of pale-sterile carmine with normal white produces a

small percentage of white offspring, which must perforce be monosomic for the chromosome containing the white locus. Such isolation by design has been practiced in a number of instances; but its utility is unfortunately seriously limited by the dearth of available simple recessive characters in the species.

As a matter of fact, however, the rate of sporadic appearance of new monosomics, especially among progenies of established ones, is so high as to have taxed our resources in studying and testing them. These sporadic variants have been the chief source of the monosomics now in our possession. Doubtless they owe their origin to the mildly non-conjunctional behavior which has been observed in many of the monosomic types.

Whatever the source, the monosomic stocks are maintained by recurrent backcrossing (monosomic ♀ × normal ♂) to the standard *Tabacum* type, Purpurea (UCBG 06-25). This method not only assures identity of the residual genotype in our stocks, but, more important, it also guards against permanent alteration in the constitution of the monosome, which in each generation is derived from the normal male parent.

Despite this method of maintenance, however, there are some modified monosomic types which may be difficult to distinguish from primary, unmodified ones. Two of the twenty-four which are now in our possession—namely, haplo-U and haplo-Z—may be considered somewhat dubious, mainly because they exhibit a relatively high frequency of non-conjunction in microsporocytes. Until more satisfactory tests than those now available can be devised, some question may remain as to the validity of some of our types, but at any rate even the dubious ones are monosomic. Whatever doubt remains resolves into a question of whether or not they are all primary types.

The maintenance of 24 monosomic types naturally enough entails considerable labor and effort in the growth of stocks and the scoring of cultures each year, particularly since many of them have relatively low transmission rates. In later years, however, with more greenhouse space available, we have found it possible to reduce the overhead materially and to maintain a full collection of the types, available at all times for such studies and crosses as may seem desirable, by propagating selected plants from cuttings. *Tabacum* cuttings root readily, particularly when treated with standard rooting agents. This procedure enables us almost indefinitely to maintain plants verified for chromosome number and tested for transmission. In view of the extraordinarily favorable reproductive features of *Tabacum*, it has always been possible to make as many crosses, sometimes a very considerable number, on such greenhouse plants as have been necessary for our purposes.

## MORPHOLOGICAL FEATURES

Monosomics differ from the standard type, Purpurea, and from one another in a specific ensemble of morphological features. In this respect they resemble trisomics, although in our experience they are not so readily recognizable as the trisomics of *Datura stramonium* or *Nicotiana sylvestris*, which is understandable, however, in view of the larger number of chromosomes and possibly

also of the dampening effect of the amphidiploid constitution of *Tabacum*. However, judged against a uniform genetic background, as has been done in these investigations, they are all sufficiently distinctive to permit reasonably accurate separation from the normal type on the basis of morphological features, which is all that is necessary for their successful employment. They differ as respects relative ease of recognition; some are very distinctive and may be scored without difficulty even if there is a considerable variation in the genetic background; others require more careful examination, sometimes with resort to auxiliary diagnostic features, presently to be described.

Although all parts of the plant are more or less distinctively affected, in actual practice classification is based chiefly upon flower features, because of their relatively greater stability under variable conditions of growth. It has been found advisable for accurate scoring to measure typical flowers from each plant, not so much for the sake of the measurements themselves as for the necessity it imposes of careful scrutiny of each plant. Many flower features have been found useful for discrimination; among them tube length and limb spread, intensity of coloration, form of the limb and of the corolla lobes, prominence of the infundibulum, style and filament length both absolute and relative, time of dehiscence of the anthers, size and character of the calyx and of the capsule, and numerous other relatively slight, quantitative features, which, although individually insignificant, together may present an ensemble of differences of sufficient magnitude to permit ready recognition. The vegetative features offer auxiliary indicia; particularly size of plant, rate of development, intensity of chlorophyll coloration, leaf shape, and character and degree of development of the auricles. The task of keeping the distinctive features of twenty-four different types in mind is not easy, especially as some of them closely resemble each other; but in any given culture, the necessary discrimination is between the normal and a single specific monosomic type, which materially simplifies classification.

## SYLVESTRIS-TABACUM HYBRIDS

When a monosomic *Tabacum* is crossed with *sylvestris* the progeny consists of two classes of hybrids, the normal 36-chromosome *sylvestris-Tabacum* hybrid and a 35-chromosome hybrid which lacks a specific *Tabacum* chromosome. It is, therefore, possible by crossing each of the monosomic types with *sylvestris* to set up a complete series of 35-chromosome hybrids corresponding to the 24 monosomic *Tabacum* types.

The 35-chromosome hybrids are readily distinguishable from the normal hybrid, and usually the morphological features of difference are more pronounced than those of the corresponding monosomic *Tabacum* types. In most cases the features of difference resemble those of the corresponding monosomics, but in a number of instances they include qualitative as well as quantitative differences. The 35-chromosome *sylvestris-Tabacum* counterparts of haplo-C and haplo-G have white instead of colored flowers, that of haplo-F has coral flower color, and that of haplo-P has pink flowers. Haplo-E *Tabacum*

is relatively slightly different from the normal type, but its 35-chromosome *sylvestris-Tabacum* counterpart is very distinct, particularly as to vegetative features. It has strongly bullate, drooping leaves, closely appressed against the main stem. Similar monosomic types may produce distinctly different *sylvestris-Tabacum* counterparts. Thus haplo-F and haplo-N resemble each other closely, but the *sylvestris-Tabacum* counterpart of haplo-F has coral flowers while that of haplo-N has intensely-colored carmine flowers. The 35-chromosome hybrids, therefore, provide an additional means of discrimination which may be extremely useful in resolving questions of doubt.

## REPRODUCTIVE FEATURES

The reproductive features of specific monosomics appear to be as distinctive as their morphological features. Some studies have been made of the distinguishing features of the monosome in microsporocyte preparations, of the behavior of the monosome in meiosis, of pollen preparations, of ovular abortion, of seed production, and of transmission ratios. The pertinent quantitative data are collected in table 1.

### *The monosomes*

In good M-I acetocarmine or acetoorcein miscrosporocyte smear preparations, the monosome is easily distinguished from the bivalents, partly by its peripheral position in the plate, partly by its shape and partly by its relative depth of staining. As a consequence it is possible to study the distinctive features of specific chromosomes. While of course the chromosomes are not well suited for revealing minute differences of morphology at this stage, it is possible to determine grosser features of size and shape, as shown by the camera lucida drawings reproduced in figure 1. The figure depicts the differences which occur among the monosomes and something as to the variation which is encountered in dealing with them. Despite the relative inferiority of this stage for studies of morphological features, it is possible to use the cytological preparations for diagnostic purposes much more effectively than would appear feasible from the figure.

### *Meiotic behavior*

Microsporocyte preparations have also been studied in order to determine the frequency and character of deviations from the normal type of association, 23II+1I. Two such types of deviation are outstanding: (1) appearance of trivalents, by association of the monosome with a bivalent—that is, 22II+1III —and (2) non-conjunctional behavior through failure of association of one or more pairs of bivalents to give cells exhibiting 22II+3I, 21II+5I, etc. The former type of behavior occurs in haplo-D and haplo-S, along with non-conjunctional behavior, to the extent of approximately 25 and 20 percent, respectively. In other monosomics trivalent associations are very rare. Non-conjunctional behavior, almost exclusively restricted to a single pair of chromosomes has been noted as occasional in haplo-J and haplo-L, about 5 percent in haplo-

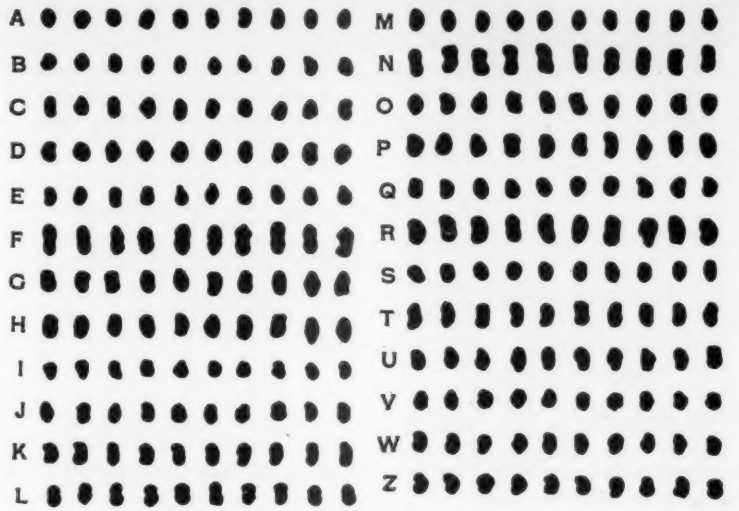


FIG. 1.—The monosomes of *Tabacum*. Ten monosomes of each type as they appear at I–M in microsporocytes.

O, about 10 percent in haplo-H and haplo-I, about 15 percent in haplo-Q, about 25 percent in haplo-U and possibly even more in haplo-Z. In the latter two, non-conjunction may occur simultaneously in two or more pairs of chromosomes, and its occurrence is reflected by production in their progenies of numerous chromosomal variants besides the expected monosomics. No doubt this phenomenon is also responsible for the relatively high sporadic production of exceptional monosomics in other monosomic progenies.

The F chromosome exhibits nucleolar association, but there is at least one other chromosome, as yet unidentified, which is also attached to the nucleolus.

### *Subgenomic classification of the monosomics*

The normal *sylvestris-Tabacum* hybrid, as has been shown by GOODSPEED (1934), exhibits 12II+12I as the modal condition for chromosome association. There are some variations in association, but not one of them approaches the frequency of the modal type which appears in about 60 percent of the microsporocytes. The 35-chromosome hybrids are of two classes: one with a modal association of 12II+11I; the other, 11II+13I; the former indicates that the missing chromosome belongs to the *tomentosa;* the latter, to the *sylvestris* subgenome of *Tabacum*. The fidelity of this behavior confirms the assumption of specificity of association of chromosomes in the hybrid upon which the original hypothesis of the amphidiploid constitution of *Tabacum* was based (CLAUSEN 1928). There are twelve types in each class. For convenience, mono-

somics involving chromosomes of the *tomentosa* subgenome have been given the letter designations A to L, and those of the *sylvestris* subgenome, M to Z. X and Y have been reserved for non-specific characterization of any monosomic or of an unknown type. On the basis of this classification a rough comparison may be made of monosomes of the two groups, by dividing them into the classes indicated below:

| | *tomentosa* subgenome | *sylvestris* subgenome |
|---|---|---|
| very small | B I | |
| small | C E | |
| medium small | A J K | Q S V W Z |
| medium | D L | M O |
| medium large | G H | T U |
| large | F | N P R |

From this grouping it is clear that the members of the *tomentosa* subgenome exhibit a wider range in chromosome size and a smaller average size than those of the *sylvestris* subgenome, but the distinction between the two does not appear to be so great as earlier studies of *tomentosa-Tabacum* and *sylvestris-Tabacum* preparations led us to expect (CLAUSEN 1932).

### *Pollen conditions*

Pollen conditions have been studied in acetocarmine preparations made by thoroughly mixing the entire contents of a single anther in a drop of the stain. Such preparations unfortunately are not permanent, but more lasting ones may be made by mounting the grains in lactophenol lightly stained with acid fuchsin. While these preparations are not so revealing as those stained with acetocarmine, they are useful to keep on hand as pollen type specimens of the monosomics.

The data in table 1 record the number of subnormal grains in the counts. This method of treatment was necessitated by the great variety of conditions exhibited by the preparations and by the difficulty of devising any hard and fast system of classification applicable to all types. In some cases the pollen grains appeared to be sharply divisible into good and aborted grains, the latter completely devoid of contents, or nearly so. Such is the case for those with high abortion rates (around 80 percent), which have been marked with an asterisk. In those with a relatively low rate of pollen abortion (A-B-C-D-E-H-S), classification was more difficult because of a gradation of pollen type which interfered with a purely objective distinction between good and bad grains. In these, only the completely aborted grains were classified as subnormal, although many other grains were obviously defective. Measurements of the good grains of preparations exhibiting high abortion revealed that they were equivalent to those of normal Purpurea; and this equivalence seemed to be complete, both as to measurements and as to condition of the contents. Measurements of good grains of those preparations with a low percentage of

TABLE 1

*Reproductive features of the monosomic types of Nicotiana Tabacum.*

| TYPE | POLLEN ABORTION | | | OVULE ABORTION | | | SEED PRODUCTION | | OVULAR TRANSMISSION | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | TOTAL | SUB-NORMAL | PER-CENT-AGE | TOTAL | ABORT-ED | PER-CENT-AGE | SEEDS PER CAPSULE | PERCENT-AGE | TOTAL | MONO-SOMIC | PER-CENT AGE |
| Pp | 922 | 30* | 3.3 | 259 | 7 | 2.7 | 2827 | 100.0 | | | |
| A | 333 | 23* | 6.9 | 457 | 82 | 17.9 | 3296 | 116.6 | 1291 | 1016 | 78.7 |
| B | 315 | 16* | 5.1 | 326 | 100 | 30.7 | 1326 | 46.9 | 1303 | 421 | 32.3 |
| C | 341 | 83* | 24.3 | 254 | 82 | 32.3 | 2354 | 83.3 | 1331 | 610 | 45.8 |
| D | 469 | 15* | 3.2 | 263 | 41 | 15.6 | 2093 | 74.0 | 835 | 344 | 41.2 |
| E | 239 | 16* | 6.7 | 263 | 77 | 29.3 | 1757 | 62.2 | 819 | 671 | 81.9 |
| F | 950 | 735 | 77.4 | 270 | 66 | 24.4 | 1646 | 58.2 | 879 | 526 | 59.8 |
| G | 452 | 342 | 75.7 | 451 | 380 | 84.3 | 535 | 18.9 | 972 | 62 | 6.4 |
| H | 453 | 101* | 22.3 | 326 | 148 | 45.4 | 2281 | 80.7 | 888 | 625 | 70.4 |
| I | 656 | 512 | 78.0 | 449 | 345 | 76.8 | 536 | 19.0 | 520 | 40 | 7.7 |
| J | 368 | 297* | 80.7 | 358 | 311 | 86.9 | 181 | 6.4 | 432 | 26 | 6.0 |
| K | 658 | 523 | 79.5 | 471 | 264 | 56.1 | 1135 | 40.1 | 381 | 184 | 48.3 |
| L | 604 | 478* | 79.1 | 398 | 290 | 72.9 | 534 | 18.9 | 183 | 34 | 18.6 |
| M | 434 | 362* | 83.4 | 291 | 95 | 32.6 | 945 | 33.4 | 803 | 480 | 59.8 |
| N | 567 | 446 | 78.7 | 346 | 274 | 79.2 | 376 | 13.3 | 784 | 173 | 22.1 |
| O | 529 | 454 | 85.8 | 306 | 50 | 16.3 | 2517 | 89.0 | 1085 | 838 | 77.2 |
| P | 579 | 446 | 77.0 | 326 | 275 | 84.4 | 739 | 26.1 | 1049 | 68 | 6.5 |
| Q | 417 | 336* | 80.6 | 241 | 191 | 79.3 | 668 | 23.6 | 845 | 93 | 11.0 |
| R | 427 | 319* | 74.7 | 284 | 131 | 46.1 | 1321 | 46.7 | 1074 | 584 | 54.4 |
| S | 466 | 58* | 12.4 | 254 | 54 | 21.3 | 2576 | 91.1 | 1093 | 339 | 31.0 |
| T | 747 | 507* | 67.9 | 316 | 271 | 85.8 | 417 | 14.8 | 437 | 48 | 11.0 |
| U | 414 | 256* | 61.8 | 322 | 238 | 73.9 | 542 | 19.2 | 231 | 84 | 36.4 |
| V | 497 | 373* | 75.1 | 665 | 530 | 79.7 | 638 | 22.6 | 242 | 19 | 7.9 |
| W | 538 | 400* | 74.3 | 546 | 430 | 78.8 | 752 | 26.6 | 234 | 12 | 5.1 |
| Z | 671 | 551* | 82.1 | 383 | 316 | 82.5 | 164 | 5.8 | 223 | 18 | 8.1 |

* Subnormal grains completely aborted, or nearly so.

complete abortion, however, revealed a wider range extending toward lower values than those of normal preparations. Some of the measurements disclosed distinctly bimodal distributions, the mode at the higher value corresponding to that of normal grains, the one at the lower level, marking the larger portion of the distribution, presumably representing the 23-chromosome grains. The remaining preparations were more or less distinctly dimorphic, with the subnormal class consisting of defective grains, distinctive for each monosomic type, marked by various features, such as smaller size, shrunken contents, or

peculiar staining of the contents, while the good ones were again equivalent in every respect to normal grains.

The studies of pollen preparations seem to show clearly that initially the microspores of each monosomic consist of 24- and 23-chromosome classes in about the proportions of 20 percent of the former and 80 of the latter. The 24-chromosome microspores develop into pollen grains equivalent in every respect to those of normal plants; but the 23-chromosome microspores develop distinctively according to their specific chromosomal constitution; in some types they abort in early development, in others they give rise to defective pollen grains, exhibiting individual features as respects degree of development and character of contents; in very few they may follow approximately a normal course of development. The individual features of the pollen preparations, therefore, depend upon the course of development of the 23-chromosome class of microspores, and they are so distinctive that they provide a very satisfactory diagnostic feature.

The conclusion from studies of pollen preparations that the percentage of 23- and 24-chromosome microspores is approximately 80 and 20, respectively, instead of 50 of each type, is closely in agreement with studies of sporogenesis. Lagging and consequent elimination of the monosome in microsporogenesis has been described by OLMO (1935). The excluded laggard chromosomes form micronuclei which may be counted at the sporad stage. OLMO's estimates for the percentage of 23-chromosome microspores, based on such counts, were 76.8 for haplo-A, 75.6 for haplo-B, 76.7 for haplo-N, and 75.0 for haplo-R. Similar studies of megasporogenesis by FANSLER (1941), a more difficult task, led to estimates of percentage of 23-chromosome megaspores of the same order of magnitude—72.8 for haplo-F, 71.1 for haplo-H, and 74.3 for haplo-O; and in a recent paper GERSTEL (1943) has shown that each of two independent, unassociated chromosomes is included in only approximately 20 percent of the megaspores. It seems to be established, therefore, that elimination of the monosome in sporogenesis occurs to practically the same extent in each monosomic type, both in microsporogenesis and in megasporogenesis, setting up a primary ratio of about 80 to 20 for 23- and 24-chromosome spores, respectively, and that the differences among the monosomic types depend primarily upon the subsequent development of 23-chromosome spores.

### *Ovule abortion*

The studies of ovule abortion are best conducted somewhat before the midpoint of the normal period from pollination to maturity of the seed, about ten to twelve days after pollination. Counts are readily made by removing a section of the outer wall of the capsule, exposing the developing ovules attached to the placental surface. Examination is conveniently made with a 20× binocular, using a small needle scoop to remove the crowded ovules as they are counted. Normal ovules at this stage are plump and have attained full size; aborted ones are mere scale-like, flattened, collapsed vestiges, still attached to the placental surface.

In contrast to the distinctiveness of pollen preparations, the studies of ovule development seem almost invariably to reveal simply a sharp distinction between ovules which appear to be developing normally and those which have aborted; but there are wide differences in percentages of aborted ovules.

The data in table 1 reveal that in a number of monosomics, twelve in fact, the percentages of abortion cluster around 80 percent, which, assuming that the 23-chromosome class of megaspores abort, is very closely in agreement with the general results of pollen studies. The remaining monosomic types exhibit ovule abortion values ranging from 15 to 56 percent.

The interpretation of the data on ovule abortion, however, is not simple. Not all of the 23-chromosome megaspores abort, even in those monosomic types which approach the 80 percent level of abortion, as is shown by their ovular transmission ratios of 5 to 20 or even more percent.

FANSLER (1942) has made some studies of ovule abortion in haplo-F, haplo-H, haplo-O, and a modified monosomic having a G-M translocation chromosome replacing a G and an M chromosome. Her results indicate that ovule abortion may arise from several causes, of which the most important were early degeneration of the endosperm, failure of megaspore development, and absence of pollination. The monosomics under study varied as to relative frequency of these phenomena. Thus the percentage of ovule abortion arising from early degeneration of the endosperm was found to be approximately 40 for haplo-H, 24 for haplo-F, and only 9 for haplo-O; but abc tion from failure of pollination occurred to the extent of about 5 and 6 percent, respectively in haplo-H and haplo-O, as contrasted with 16 percent in haplo-F. There was some slight evidence of a modified Renner effect, as seen in occasional development of megaspores other than the chalazal one into embryo sacs. This phenomenon, however, occurred too infrequently, not more often than four percent in haplo-H, to be an important factor in the situation, and when it occurred it seemed to be a random matter as to which of the other three megaspores would develop. Unfortunately these studies did not include any representative of the class of monosomics exhibiting high ovule abortion. The mid-development counts reported in table 1 appear to be equivalent to abortion arising from endosperm degeneration, and the extent to which this phenomenon is selective is debatable, since haplo-H in which 45 percent of the ovules abort, still has a transmission rate approaching the maximum value, whereas haplo-S which has only 21 percent of ovule abortion, nevertheless shows a much more marked depression in the transmission rate. High-abortion monosomics, however, all have low transmission rates. The phenomenon of abortion as a consequence of endosperm degeneration is reminiscent of BRINK and COOPER'S (1941) somatoplastic sterility; but further investigations are needed to determine the extent to which it is selective. At any rate, it is clear that maximum values approach 80 percent as with pollen abortion and that the high-abortion rates are associated with low transmission of the monosomic condition.

*Seed production*

Seed-production values, entered in table 1, have been obtained from capsules from hand-pollinated flowers. The seeds from single capsules were weighed; the number of seeds per capsule was estimated by counting the number of seeds in a ten milligram sample. Naturally most of the monosomics produce fewer seeds per capsule than normal plants; but one, haplo-A, appears to produce more. On the whole, seed production is reasonably closely correlated with percentage of good ovules, especially in the group of plants with high ovule abortion, but the relations among the remaining types are rather erratic. The method of estimation of ovular abortion may be at fault, since the determinations were made in the broadest portion of the capsule near the base. There was some evidence of uneven distribution of ovular abortion in the capsules. Moreover the comparison makes the tacit assumption that potentially all types produce the same number of ovules, which certainly has not been proved.

*Ovular transmission*

The ovular transmission rates recorded in table 1 have all been obtained from crosses of monosomic ♀ × normal ♂. These crosses may at the same time have included differences in Mendelian characters, but crosses between distinct varieties, which usually exhibit hybrid vigor, have been excluded. The populations here included have all been grown from counted samples of seed germinated on filter paper, the seedlings then transferred to pots, and from them successively to flats and to the field. These precautions were taken in order to eliminate selective effects among seedlings, which previous experience with ordinary methods of propagation has shown to be very marked for some monosomic types. Germination percentages of the seeds of the various monosomic types are astonishingly high and do not differ significantly among themselves nor from normal seed. Despite all precautions, however, the percentage of transmission of most of the monosomic types exhibits a high range of variation, far too high to be ascribed simply to random sampling. Apart from this unresolved difficulty, each monosomic is characterized by a specific transmission rate. The range of difference extends from 5 to above 80 percent. The maximum values are attained in only a few types, notably A, E, and O, in which the monosomics apparently appear in the progenies in about the same proportion as that in which the 23-chromosome megaspores are produced. Particularly notable, however, is the fact that those monosomics which belong to the high ovular abortion class, still exhibit some transmission, thus making it possible to propagate them and to work with them.

*Correlative behavior*

In figure 2 we have attempted to show graphically the degree to which correlative behavior exists with respect to pollen abortion, ovule abortion, seed production, and ovular transmission. The data have been taken from table 1,

but some slight shifts in values have been made in order to prevent confusion of lines in the figure.

The main conclusion which may be drawn from this figure is that there is a group of 12 monosomics (G-I-J-L-N-P-Q-T-U-V-W-Z) which is set apart from

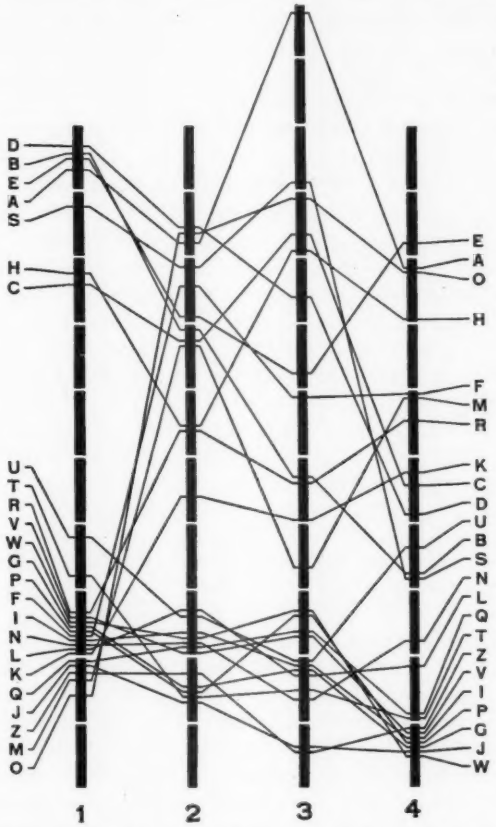


FIG. 2.—Relations among the monosomic types as to (1) percentage of good pollen, (2) percentage of good ovules, (3) seed production in percentage of normal set, and (4) ovular transmission rate from monosomic ♀ × normal ♂. The vertical scales are interrupted at 10 percent intervals. Data from table 1.

the remaining types by low percentage of good pollen and ovules, low seed production, and low ovular transmission rates. There is a good deal of overlap as respects pollen conditions, since the halpo-types, F, K, M, O, and R are also listed as showing low percentages of good pollen; but in F, K, and O this classification is based upon a dimorphic condition of the pollen, rather than upon

complete abortion of the 23-chromosome grains. However, it is established that although pollen and ovular conditions are usually parallel, they are not necessarily so. Aside from that in pollen conditions, the two groups exhibit no further overlapping, except for the rather high recorded ovular transmission rate of haplo-U, which may depend in part on paucity of data for this type; more likely it depends upon inaccurate classification arising from confusion with variant types which appear in relatively high frequency in its progenies. Haplo-Z also produces a high percentage of variant types, but since its morphological features are more distinctive, there is less confusion in its classification.

In the other group of twelve types, there is no very pronounced correlative behavior within the group.

## MONOSOMIC ANALYSIS

In monosomic analysis, associations in transmission of monosomic types with specific Mendelian factors replace the customary Mendelian ratios as evidence of the relations among factors and chromosomes. The method is not new, for it is exemplified in its essential features in sex-linked inheritance, where the heterogametic sex is monosomic for the sex chromosomes and hence hemizygous for factors located in it. With a complete set of monosomics, the well-known advantages of sex linkage in the study of genetic relations become applicable, at least in part, to all chromosomes of the set. The simplification accomplished by monosomic analysis is especially important in species with high chromosome numbers, since it permits accomplishment with them of something in the way of chromosomal analysis comparable to that which has been done with low-numbered species. More important still, it may make it possible to attack with some degree of precision the general problem of the genetic constitution of polyploid species.

### *Mendelian factors in Tabacum*

Unfortunately a large number of simple Mendelian characters is not available in *Tabacum*, in which respect *Tabacum* appears to be a typical polyploid species. Those in our possession have mostly been transferred to our standard variety, Purpurea, by the method of recurrent backcrossing in order to have an identical residual background in our tests. This is not absolutely necessary for application of monosomic analysis, but it is at least advantageous, since complex segregation for quantitative features may interfere seriously with accurate classification of the monosomics.

The factors which have been tested thus far for chromosomal association are listed below. In assigning symbols to them, the convention has been followed of naming the factor from the character difference which it sets up from our standard variety, Purpurea; symbols with initial capitals represent dominant and those with lower-case initials represent recessive factors.

*Br-* broad: broad leaf base as contrasted with the sharply constricted leaf base of the standard type. The heterozygote is intermediate, but it is most convenient to include homozygous and heterozygous broad in one class.

*co-* coral: salmon-pink flower color associated with dwarf stature and small

grayish-green leaves. Coral is a deficiency character arising from substitution of a segment of the N chromosome for a segment of the non-satellited arm of the F chromosome.

*cy-* calycine: a teratological flower type with more or less extremely split corollas and enlarged, partially petaloid calyces.

*hf*1- *hf*2- hairy filament: a duplicate factor character from NOLLA's (1936) variety, Ceniza.

*lf-* light filaments: faintly colored filaments as contrasted with the dark filament color of Purpurea. Expression of the character is limited to plants having carmine flower color; pink and white flowering plants always have light filament color.

*Ml-* many leaved: a dominant character, transferred from *tomentosa*, marked by increase of five or six in leaf number. The heterozygote has the same leaf number as the homozygote.

*mm-* mammoth: the well-known growth type which exhibits marked photoperiodic response; indeterminate leaf number under long-day and determinate under short-day conditions, incompletely recessive to the normal growth type. Our mammoth race was established by transfer of the mammoth character from the commercial variety, Maryland Mammoth, to Purpurea.

*pa-* pale-sterile: a partially asynaptic type exhibiting an average of about eleven bivalents per microsporocyte; the rest of the chromosomes unassociated. Highly sterile, pollen almost completely aborted, seed production from pale-sterile ♀ × normal ♂ about five percent of the normal yield per capsule. Pale-sterile is completely recessive and must be carried along by selection of heterozygous plants.

*Pd-* petioloid: a petioled type with narrow, acutely pointed leaves and other associated features. Resembles petioled (*q.v.*) but is not so extreme either as to length of petiole or narrowing of the leaf. In combination with petioled, it produces a very extreme leaf type with a long petiole and long, very narrow, pointed blade.

*pk-* pink: light pink flower color as contrasted with carmine, to which it is almost completely recessive.

*Pp-* purple-plant: a dominant factor transferred from *tomentosa*. Stems, leaves, calyx and immature capsules exhibit a conspicuous, bronze-purple color. It requires exposure to sunlight for full development, but the associated purple style color seems to develop without such exposure.

*Ps-* purple-style: the style only has a more or less pronounced purple color. It should not be confused with the purple style color of purple-plant, which is inseparably associated with that character-complex.

*Pt-* petioled: this factor produces a complex of differences from normal, of which the narrow, petioled leaf type is most conspicuous. Associated differences include narrow, long-pointed calyx lobes, long-pointed corolla lobes, long, narrow, pointed capsules, semi-dwarf stature, and basal branching habit. The heterozygote is somewhat intermediate, but difficult to separate accurately from the homozygote.

*rd-* red: a dark modifier of pink such that *pk rd* has a dark red flower color almost equivalent to carmine. The heterozygote is pink, but more vividly colored than homozygous pink.

*sg-* stigmatoid: malformed anthers frequently tipped with a miniature style and stigma, together with associated plant features, particularly a moderate dwarfing of the plant and a reduction in flower size. The heterozygote is slightly intermediate as to flower size, but otherwise it is completely normal in expression. It is one of the X-ray types produced by GOODSPEED (1930).

*wh-* white: white as contrasted with colored flowers.

*wh-P-* pale: a flower color character; pale limb color, faintly colored tube and filaments. An allele of white transferred from *tomentosa*. It is recessive to full color but dominant to white.

*ws-* white-seedling: the seedlings are apparently totally devoid of chlorophyll and die soon after germination. It also is an X-ray type produced by GOODSPEED (1930).

*yb*$_1$- *yb*$_2$- duplicate factors for yellow burley, the familiar commercial character.

*yg-* yellowish-green: the partially chlorophyll-deficient character of NOLLA's (1934) variety, Consolation. A simple recessive to normal green.

The simplest genetic problem to which monosomic analysis may be applied is that of determining the location of factors in chromosomes. The type method is illustrated by results previously published in an earlier paper (CLAUSEN and GOODSPEED 1926b), as follows:

$P_1$ haplo-C carmine ♀ × diplo-C white ♂
$F_1$ haplo-C white + diplo-C carmine

The $F_1$ ratio is of no importance to the demonstration, it is the association of haplo-C with white which demonstrates that the factor for white is located in the C chromosome. A similar cross with each of the other 23 monosomic types gives an $F_1$ progeny which is carmine in both the monosomic and the normal classes, which just as positively demonstrates that the white factor is not located in any one of the other chromosomes.

The standard procedure for determining an association then is to cross the normal recessive with each of the 24 monosomic dominants, whereupon the results in general will be as follows:

$P_1$ haplo-X dominant × diplo-X recessive
$F_1$ haplo-X recessive + diplo-X dominant

if the factor is located in the chromosome, or if it is not:

$F_1$ haplo-X dominant + diplo-X dominant

The simplicity of the test and its advantages, as compared with the trisomic and other methods of determining such relations are so obvious as not to require elaboration. If dominant characters are to be tested, the dominant type is first crossed with each monosomic, in order to establish the necessary haplo-X dominant types, whereupon association is determined in the second generation

according to the pattern described above. In the case of complex factor situations, double recessives, homozygous lethals, complementary factors, etc., simple modifications of the procedure may easily be devised in order to establish associations, as is shown by some of the examples described below.

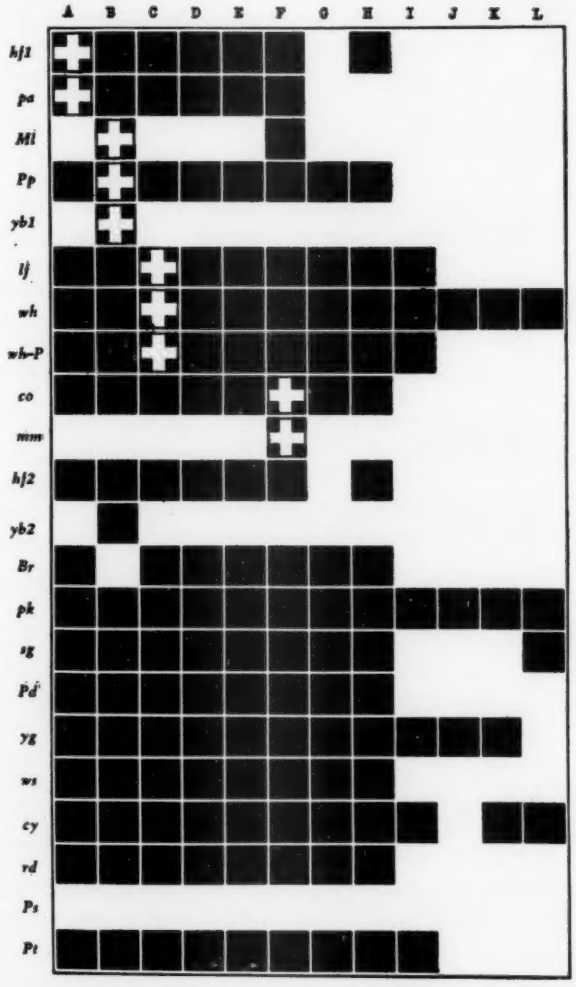


FIG. 3a.—Tests of factor associations with haplo-types of the *tomentosa* subgenome. The plus squares represent positive associations, the black squares negative results, and the blank spaces untested relations. Capital letter captions at the top represent chromosomes; italic letters at the left side, factor symbols as listed in the text.

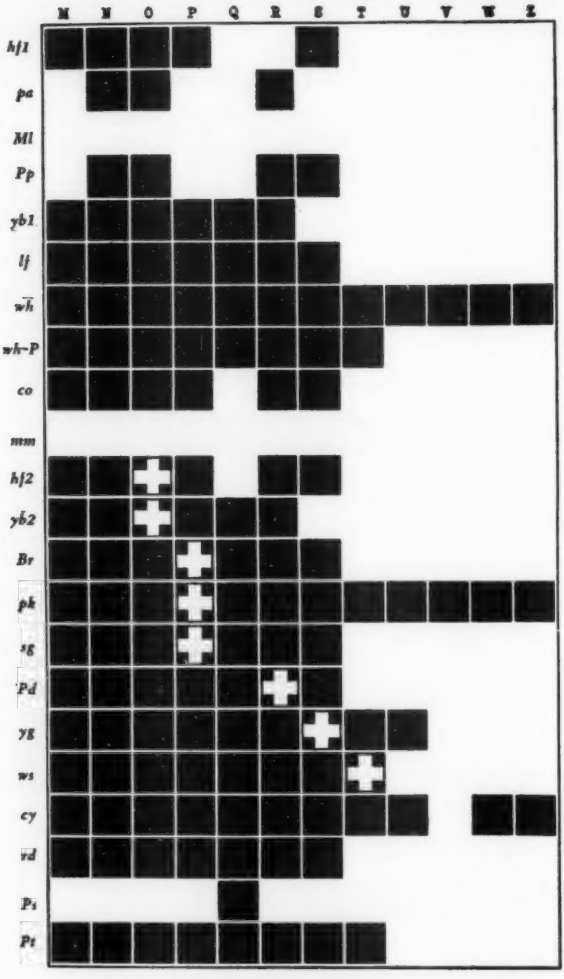


FIG. 3b.—Tests of factor associations with haplo-types of the *sylvestris* subgenome. Significance as in Figure 3a.

The results of 294 test crosses for association of factors with chromosomes are assembled in two diagrams (fig. 3a, 3b), in which a plus square indicates positive association; a black square, absence of association; and a blank space, an untested relation. In practice, tests for association are discontinued as soon as a positive association has been established.

The figure shows that the following factors have been located in specific chromosomes:

| | | | | | |
|---|---|---|---|---|---|
| A | *hf*1 | —hairy-filament | | *mm* | —mammoth |
| | *pa* | —pale-sterile | O | *hf*2 | —hairy-filament |
| B | *Ml* | —many-leaved | | *yb*2 | —yellow-burley |
| | *Pp* | —purple-plant | P | *Br* | —broad leaf-base |
| | *yb*1 | —yellow-burley | | *pk* | —pink flower color |
| C | *lf* | —light-filament | | *sg* | —stigmatoid |
| | *wh* | —white flower color | R | *Pd* | —petioloid |
| | *wh-P* | —pale flower color | S | *yg* | —yellowish-green |
| F | *co* | —coral flower color | T | *ws* | —white-seedlings |

## SPECIFIC ASSOCIATIONS

The following accounts of results of tests for associations are, for the most part, limited to positive instances, since it seemed unnecessary to report details of negative results beyond the specific enumeration of the tests conducted, which are recorded in figures 3a and 3b. The details of positive associations are presented individually in order to illustrate the modifications of the type procedure which are necessary in order to meet specific genetic conditions.

### *Association of hairy-filament with haplo-A and haplo-O*

Hairy-filament is a character exhibited by the variety Ceniza, described and distributed by NOLLA (1936). Acting under assumption that hairy-filament is a simple recessive character, we crossed our haplo-types with Ceniza and found in one case—namely, with haplo-A—that the haplo-A plants exhibited the character, but very weakly. The weak expression of the character might have depended upon the difference of genetic background, but when these haplo-A plants were selfed, they exhibited a ratio of approximately 3 normal: 1 hairy-filament, provided the hairy-filament class was restricted to those plants which showed a strong expression of the character. Sister diplo-A $F_1$ plants gave $F_2$ segregation approximating 15 normal: 1 hairy-filament. These results demonstrated that our variety Purpurea contained duplicate genes for the normal condition.

In view of these preliminary results, a further set of tests was conducted in order to determine the location of the second gene, since the preliminary results had shown one of them, *hf*1 to be located in the A chromosome. These tests were started by crossing the available haplo-types with hairy-filament, then selected $F_1$ haplo-types heterozygous for hairy-filament were selfed for production of $F_2$.

| | smooth-filament | hairy-filament |
|---|---|---|
| A 41323 | 25 | 15 |
| B 41325 | 47 | 1 |
| C 41326 | 44 | 4 |

| | | | |
|---|---|---|---|
| D | 41327 | 40 | 2 |
| E | 41328 | 33 | 2 |
| G | 41329 | 36 | 0 |
| H | 41330 | 38 | 2 |
| M | 41333 | 39 | 5 |
| N | 41334 | 41 | 1 |
| O | 41335 | 30 | 15 |
| P | 41336 | 37 | 2 |
| S | 41339 | 37 | 1 |
| Totals (excluding A and O) | | 394 | 20 |
| Expectation 15:1 | | 388 | 26 |

These results point to A and O as the chromosomes in which *hf*1 and *hf*2 are located, as is evidenced by the high proportion of hairy-filament segregants in the progenies of the $F_1$ haplo-types heterozygous for hairy-filament. Division of these progenies into normal and haplo-types has not been recorded in the table because it furnished no pertinent information as to location of the genes. Classification for the haplo-types was in some instances very uncertain, because of the considerable degree of segregation occurring in $F_2$ of crosses between Purpurea and Ceniza, the source of the hairy-filament character. Segregation for hairy-filament, however, was independent of the haplo-type division. Excluding A and O, the segregation is in satisfactory agreement with the 15:1 ratio for duplicate genes.

In the course of these tests it was noted that haplo-O heterozygous for hairy-filament exhibited a weak expression of the character, similar to that previously noted for haplo-A heterozygous for hairy-filament, and that apparently some other heterozygous conditions might likewise do so. The extent and the basis of these weak expressions have not as yet been entirely clarified, but in the homozygous condition the hairy-filament character is even more pronounced in our Purpurea derivatives than in Ceniza.

For a more complete exposition of the analytical features of the monosomic system of locating duplicate genes, the reader is referred to the account of yellow burley, following.

*Association of pale-sterile with haplo-A*

$P_1$ haplo-A normal ♀ × diplo-A pale sterile heterozygote ♂

| | | haplo-A pale-sterile | haplo-A normal | diplo-A normal |
|---|---|---|---|---|
| $F_1$ | 33128 | 17 | 5 | 6 |
| | 36170 | 7 | 21 | 12 |
| | 36253 | 12 | 18 | 8 |
| | 37238 | 16 | 21 | 5 |
| | Totals | 52 | 65 | 31 |

These results illustrate the method to be employed when it is not possible to use the homozygous type in the tests. Pale-sterile plants exhibit almost com-

plete pollen abortion, and their seed progeny is so variable, on account of the disturbed distribution of chromosomes, that it is impracticable to study transmission of the character except through employment of heterozygous plants.

*Association of many-leaved and purple-plant with haplo-B*

| | | |
|---|---|---|
| $P_1$ | | haplo-B many-leaved purple-plant ♀ × |
| | | diplo-B normal-leaved green-plant ♂ |
| $F_1$ | 37240 | 22 haplo-B normal-leaved green-plant |
| | | 13 diplo-B many-leaved purple-plant |

As noted in the list of factors, both of these factors have been transferred from *tomentosa* by the method of recurrent backcrossing. The transfer of the many-leaved type was an unexpected consequence of the result of transferring the more-obvious purple-plant character to our standard variety of *Tabacum*. After the transfer of the purple-plant factor had been accomplished it was noticed that the purple-plant type was taller and somewhat later than the standard type, and counts showed that it had five or six more leaves than the normal number. Linkage between the two factors is very close; to date backcrosses of heterozygous many-leaved purple-plant to normal-leaved green-plant have given only 3.6 percent of recombinations, as shown by the following results from seven such progenies: 125 many-leaved purple-plant, 144 normal-leaved green-plant, 8 many-leaved green-plant, 2 normal-leaved purple-plant.

*Association of yellow-burley with haplo-B and haplo-O*

The inheritance of yellow-burley of commercial burley varieties, as originally demonstrated by KAJANUS (1924) and more recently confirmed by HENIKA (1932), is dependent upon duplicate genes, so that $F_2$ ratios approximate 15 green: 1 yellow-burley. These results agree with our own determinations which employed the commercial variety Station Standup (MACRAE and HASLAM 1935) as the source of yellow-burley and our standard variety Purpurea as the green parent. The monosomic analysis demonstrates that one of the factors, *yb*1, is located in the B chromosome; the other, *yb*2, in the O chromosome.

The *yb*1 association with haplo-B is demonstrated by the following series of results. Haplo-B green ♀ × diplo-B yellow-burley ♂ gives two classes of $F_1$ offspring—haplo-B green and diplo-B green—both heterozygous for yellow-burley. These two classes of plants were then tested for yellow-burley segregation in progenies from selfing and from backcrosses to yellow-burley.

The results from $F_1$ diplo-B green plants selfed:

| $F_1$ | diplo-B yellow-burley heterozygote × self | | |
|---|---|---|---|
| | | diplo-B green | diplo-B yellow-burley |
| $F_2$ | 41310 | 43 | 3 |
| | 41319 | 36 | 3 |
| | Totals | 79 | 6 |

And from the same plants backcrossed to yellow burley:

$F_1$ diplo-B yellow-burley heterozygote ♀ × yellow-burley ♂

| | | diplo-B green | diplo-B yellow-burley |
|---|---|---|---|
| Backcross | 41312 | 34 | 10 |
| | 41321 | 30 | 10 |
| | Totals | 64 | 20 |

The results from selfing are in satisfactory agreement with 15:1 duplicate-gene segregation, and those from backcrosses are in agreement with the expected 3:1 ratio.

The tests of sister haplo-B plants for yellow-burley segregation on the other hand conform to the 3:1 ratio for selfing and the 1:1 ratio for backcrossing as shown by the following results:

$F_1$ haplo-B yellow-burley heterozygote × self

| | | diplo-B green | haplo-B green | diplo-B yellow-burley | haplo-B yellow-burley |
|---|---|---|---|---|---|
| $F_2$ | 41313 | 10 | 26 | 4 | 7 |
| | 41316 | 15 | 15 | 7 | 5 |
| | Totals | 25 | 41 | 11 | 12 |

$F_1$ haplo-B yellow-burley heterozygote ♀ × yellow-burley ♂

| | | diplo-B green | haplo-B green | diplo-B yellow-burley | haplo-B yellow burley |
|---|---|---|---|---|---|
| Backcross | 41315 | 8 | 7 | 5 | 15 |
| | 41318 | 25 | 3 | 14 | 4 |
| | Totals | 33 | 10 | 19 | 19 |

The following reciprocal backcross offers further confirmation of 1:1 segregation:

diplo-B yellow-burley ♀ × haplo-B yellow-burley heterozygote ♂

| | | diplo-B green | diplo-B yellow-burley |
|---|---|---|---|
| Backcross | 41314 | 18 | 19 |
| | 41317 | 18 | 22 |
| | Totals | 36 | 41 |

The significance of these results is apparent from the following factorial analysis:

haplo-B green $\frac{B - Yb_1}{B_0}\ \frac{O - Yb_2}{O - Yb_2}$

diplo-B yellow-burley $\frac{B - yb_1}{B - yb_1}\ \frac{O - yb_2}{O - yb_2}$

haplo-B green ♀ × diplo-B yellow-burley ♂:

$F_1$ haplo-B green $\frac{B - yb_1}{B_0}\ \frac{O - yb_2}{O - Yb_2}$

diplo-B green $\frac{B - yb_1}{B - Yb_1}\ \frac{O - yb_2}{O - Yb_2}$

The $F_1$ haplo-B plants are heterozygous for *Yb2 yb2* only, whereas their diplo-B sister plants are heterozygous for both pairs of factors. In these formulae, the convention Bo is employed to represent the missing B chromosome in haplo-B.

For determining the chromosome which carries *yb2*, the assumption was made that inasmuch as *yb1* is located in one of the chromosomes of the *tomentosa* subgenome, the chances were good that *yb2* would be located in a member of the *sylvestris* subgenome. Consequently initial tests were confined to the haplo-types then available for that subgenome. The haplo-types were first crossed with yellow-burley, then selected $F_1$ haplo-types heterozygous for yellow-burley were backcrossed to yellow-burley. Evidently *yb2* is located in the O chromosome, as shown by the results recorded below:

| | | green | yellow burley | percentage yellow burley |
|---|---|---|---|---|
| M | 42397 | 36 | 9 | 20 |
| N | 42398 | 28 | 8 | 22 |
| O | 42399 | 19 | 17 | 47 |
| P | 42400 | 33 | 9 | 21 |
| Q | 42401 | 32 | 12 | 27 |
| R | 42402 | 27 | 12 | 31 |
| S | 42403 | 27 | 4 | 13 |
| T | 42404 | 28 | 8 | 22 |
| U | 42405 | 37 | 8 | 18 |
| Totals (exluding haplo-O) | | 248 | 70 | 22 |

These results illustrate the modifications in procedure necessary to employ monosomic analyses in the location of duplicate genes. However, they do not illustrate all the advantages which may be gained from it. Thus, if it be desired to establish types homozygous for each dominant gene singly, a direct method is available. Haplo-B green × diplo-B yellow-burley, followed by selfing

of the $F_1$ haplo-B plants and selection for green will establish the *yb1 yb1 Yb2 Yb2* type, and *mutatis mutandis* the *Yb1 Yb1 yb2 yb2* type may be produced from haplo-O green×diplo-O yellow-burley. The advantages are evident from consideration of the number of tests which must be made in order to insure establishment of these two monogenic green types by the traditional method. We hope to present later a more complete demonstration of monosomic-analysis of yellow-burley inheritance.

*Association of light-filament with haplo-C*

$P_1$ haplo-C dark-filament ♀ ×diplo-C light-filament ♂
$F_1$ 38252 29 haplo-C light filament
12 diplo-C dark-filament

The demonstration here conforms to the standard procedure. As noted in the description of the character, light-filament is only determinable in carmine plants. Most *Tabacum* varieties have pink flowers and as a consequence light filaments. To determine whether or not they are genetically light-filament, they may be crossed with haplo-C carmine, as above, and the presence of light or dark colored filaments in association with haplo-C will immediately reveal their genetic constitution with respect to this feature

*Association of white and pale with haplo-C*

Numerous demonstrations of association of white and pale are available of which the following are illustrative:

$P_1$ haplo-C carmine ♀ ×diplo-C white ♂
$F_1$ 36092 21 haplo-C white+14 diplo-C carmine
37242 23 haplo-C white+20 diplo-C carmine

Association of pale with haplo-C is demonstrated by the following results:

$P_1$ haplo-C carmine ♀ ×diplo-C pale ♂
$F_1$ 33136 25 haplo-C pale+7 diplo-C carmine
34082 19 haplo-C pale+7 diplo-C carmine

Like white, pale is recessive to carmine and segregates in $F_2$ in a simple ratio. When it is crossed with white, $F_1$ is pale, and $F_2$ gives a simple ratio of 3 pale: 1 white. Pale, therefore, is an allele of white. However, since pale is a transferred character derived from *tomentosa*, it possibly may not differ so simply from carmine and white as the formulation here adopted suggests, for the *tomentosa* segment in which it was transferred may differ in a number of factors from those in the corresponding *Tabacum* segment.

*Association of coral with haplo-F*

| $P_1$ haplo-F carmine ♀ +diplo-F coral ♂ | haplo-F coral | diplo-F carmine |
|---|---|---|
| $F_1$ 34164 | 7 | 37 |
| 35208 | 5 | 23 |
| 35209 | 11 | 11 |
| Totals | 23 | 71 |

Haplo-F coral plants are difficult to raise, especially under field conditions, so that, as in these cultures, the number of haplo-F plants is far below expectation. The evidence that coral is a deficiency character comes from both cytological and genetical studies and will be presented in detail in a subsequent paper. Briefly, diplo-F coral sometimes has multivalent associations involving as many as four chromosomes; and, although haplo-F almost always is 23II +1I, haplo-F coral exhibits 22II+1III in about one-fourth of the microsporocytes. Moreover, in haplo-N plants, containing a normal N chromosome, a normal F chromosome and a coral F chromosome, the chromosomes are associated in a chain trivalent in about two-thirds of the microsporocytes. From these and other considerations we may conclude that coral has a normal N chromosome, but that its F chromosome has become altered through exchange of a segment for a segment of the N chromosome.

*Association of mammoth with haplo-F*

$P_1$ a) haplo-F normal-growth ♀ × diplo-F mammoth ♂
b) haplo-F normal-growth ♀ × haplo-F mammoth ♂
$F_1$ a) 33334 22 haplo-F mammoth+8 diplo-F normal-growth
b) 35213 23 haplo-F mammoth+6 diplo-F normal-growth

These results are to be compared with those for coral listed above.

The relation between mammoth and haplo-F was one of the first to be established. It immediately suggested an efficient method of transferring a recessive character from one variety to another, which was applied to the transfer of the mammoth character from Maryland Mammoth to Purpurea, as follows:

$P_1$ haplo-F Purpurea ♀ × Maryland Mammoth ♂
$F_1$ haplo-F mammoth + diplo-F normal
$B_1$ haplo-F Purpurea ♀ × $F_1$ haplo-F mammoth ♂

followed by repetition of the backcross in successive generations until the Maryland Mammoth features were completely eliminated. Since each generation is backcrossed, progress is twice as rapid as it is when each generation of backcrossing is separated by a selfed generation for obtaining mammoth segregants, according to the customary program of recurrent backcrossing.

*Association of broad with haplo-P*

$P_1$ haplo-P constricted carmine ♀ × haplo-P broad pink ♂
$F_1$ 38159 0 haplo-P broad pink
34 diplo-P broad carmine

The direct demonstration of the association of broad with haplo-P by means of a test cross of the following type,

$P_1$ haplo-P broad ♀ × diplo-P constricted ♂
$F_1$ haplo-P constricted + diplo-P broad

cannot very well be employed, because haplo-P constricted itself has a broad leaf base as a feature of its haplo-P condition. A further generation would

demonstrate the genetically constricted constitution of these haplo-P plants since their diplo-P offspring would all be constricted, but this test has not been made. The present test, however, is just as satisfactory. The haplo-P broad pink plant was obtained from the culture listed below, the offspring of a cross of haplo-P constricted carmine ♀ × diplo-P broad pink ♂. The fact that the male gametes of this plant all carried the broad factor demonstrates that it is located in the P chromosome. The total absence of the expected haplo-P broad pink plants does not affect the validity of the demonstration.

*Association of pink with haplo-P*

$P_1$ haplo-P constricted carmine ♀ × diplo-P broad pink ♂
$F_1$ 37245 4 haplo-P broad pink
36 diplo-P broad carmine

Since broad and pink are both located in the P chromosome, they should exhibit linkage relations. The recombination value actually obtained in experiments conducted for this purpose was approximately 4.8 percent, as shown from the following summarized totals from fourteen progenies of broad pink/constricted carmine heterozygotes backcrossed to constricted pink: 289 constricted carmine, 272 broad pink, 15 broad carmine, 13 constricted pink.

It is also interesting to note that, just as a transfer from *tomentosa* was shown simultaneously to involve two linked factors, so one of broad from *sylvestris* to *Tabacum* included a factor for pink, although its presence was not revealed until the derivative lines were self-fertilized in order to obtain homozygous broad. These characters, however, already existed among our *Tabacum* stocks, whereas those from *tomentosa* represent new features in *Tabacum*.

*Association of stigmatoid with haplo-P*

$P_1$ a) haplo-P normal-anther ♀ × diplo-P stigmatoid ♂
b) haplo-P normal-anther ♀ × haplo-P stigmatoid ♂
$F_1$ a) 37256 2 haplo-P stigmatoid + 39 diplo-P normal-anther
b) 38160 2 haplo-P stigmatoid + 31 diplo-P normal-anther

Experiments to determine the linkage relations of stigmatoid with broad and pink are in progress, but results are not yet available.

*Association of petioloid with haplo-R*

$P_1$ haplo-R petioloid ♀ × diplo-R normal ♂
$F_1$ 42371 28 haplo-R normal
18 diplo-R petioloid

The monosomic demonstration for a simple dominant requires two generations. The above tests started with a cross of each of the available monosomic types with diplo-X petioloid. From the $F_1$, haplo-X petioloid plants were selected and tested as above; the results revealed association of petioloid and haplo-X. They also might have been tested by selfing, which in view of the

hemizygous condition of R-chromosome factors would produce diplo-R plants homozygous for all of their R-chromosome factors.

*Association of yellowish-green with haplo-S*

$P_1$ haplo-S normal ♀ × diplo-S yellowish-green ♂
$F_1$ 42372 20 haplo-S yellowish-green
24 diplo-S normal

The demonstration for yellowish-green leaf color follows the standard procedure precisely. The results confirm those of NOLLA (1934) as to the simple recessive basis of yellowish-green.

*Association of white-seedling with haplo-T*

$P_1$ haplo-T normal ♀ × diplo-T white-seedling heterozygote ♂
39097p6 × 146p4 1086 green seedlings
56 white seedlings

This test for white-seedling association essentially follows the pattern for pale-sterile described previously, inasmuch as green plants heterozygous for white-seedling must be used as the source of that character. In the above progeny, the white seedlings are haplo-T, and presumably an equal number of haplo-T green seedlings were present, consequently the 4.90 percent of white seedlings indicates a transmission rate of 9.8 percent for haplo-T, which is in satisfactory agreement with the value 11.0 recorded in table 1.

Haplo-T is an illustration of a monosomic isolated by design as the specific chromosome in which *ws* is located. The original tests revealed that *ws* was not located in any of the chromosomes for which monosomics were then available. The isolation of the desired monosomic was then accomplished by the following procedure. First, pale-sterile ♀ × green heterozygous for white-seedling ♂ gave seeds which were tested for white-seedling segregation. Presence of a few white seedlings indicated that the monosomic was viable. From a further cross of pale-sterile ♀ × calycine ♂, two populations were grown, together comprising 40 plants. These plants were crossed individually with a plant known to be heterozygous for white-seedling and at the same time also with normal Purpurea. Seedling tests of the former crosses disclosed two plants which produced some white seedlings. Populations were then grown from the crosses of these parent plants with normal. Both exhibited complex segregation, but in one of them a new haplo-type with distinctive features was recognized. Subsequent tests proved that it was monosomic for the chromosome in which *ws* was located, as was to be expected. Calycine was here employed for the sake of economy in the hopes of obtaining the type monosomic for the chromosome in which *cy* is located; but although one of the $F_1$ plants was calycine, hence presumably monosomic for this chromosome, it was of such complex constitution chromosomally that through some mischance we failed to identify the desired monosomic in its progeny.

*Incomplete tests for association*

The diagrams show that four genes—*cy*, calycine; *rd*, red; *Ps*, purple-style, and *Pt*, petioled—so far have given negative results for tests of association to which they have been subjected. Tests for none of them, however, have been completed. If it should prove that any one of them or of other factors now undergoing tests should fail to exhibit association with one of the twenty-four monosomic types, it would constitute *prima facie* evidence that our collection of monosomics did not contain all the primary types, but it would not show which particular one was at fault. The demonstration of a positive association, on the other hand, provides final proof of acceptability of a monosomic as a primary, unmodified type.

## EXCEPTIONAL PROGENY

As we have noted previously, monosomics are a fertile source of variant chromosomal types. Such types are usually most effectively recognized in selfed progenies, but sometimes they appear also in analytical progenies of the kind reported in this series of investigations.

Occurrence of variegation types, presumably through spontaneous production of unstable ring chromosome fragments, has been recorded for the F chromosome, giving carmine-coral variegation (CLAUSEN 1930); for the B chromosome, giving purple-plant-green plant variegation; for the C chromosome, carmine-white (STINO 1940) and for the P chromosome, carmine-pink. Apart from the fact that they all exhibit the same general type of variegation—that is, dependent upon sporadic elimination of the dominant color factor during ontogeny—they are very diverse as respects transmissibility, stability and types of derivative products. Each one poses a cytogenetic problem of some complexity.

Other products which have been recognized and studied to some extent include fragment products of the monosome. In the case of the C chromosome two small fragments, one of which appears to be equivalent mainly to one arm of the chromosome and one to the other arm, appear rather frequently. A stock has been set up in which these two complementary fragments have been substituted for the normal C chromosome (STINO 1938). It consequently exhibits 25 II chromosomes in meiosis and breeds true for this condition. Morphologically it differs only in minor respects from the normal type.

Perhaps the most significant recurrent aberrancy which has been determined is an oscillating relation between monosomics and their trisomic counterparts. Crosses of monosomic ♀ × normal ♂ give a small percentage of trisomic counterparts; and similarly those of trisomic ♀ × normal ♂ in turn produce occasional monosomics. This oscillating disturbance in distribution of chromosomes provides a convenient way of producing the counterpart types and of matching them up. Haplo-C and triplo-C, haplo-F and triplo-F, haplo-N and triplo-N, and haplo-R and triplo-R have been observed to behave according to this pattern, and no doubt the others do also. It is not surprising to find that the morphological features of these trisomics differ from normal

in the opposite direction from that of their monosomic counterparts. Haplo-F and haplo-N are small-flowered types and triplo-F and triplo-N have large flowers; haplo-C which is large-flowered produces triplo-C which is distinctly small-flowered. This oscillating relation may also exist in basic species. EINSET (1943) has recently reported the presence of monosomics in trisomic progenies of maize, but their specific constitutions were not determined. The production of trisomics from monosomics, however, apparently presents some complications. About half of a number of independent instances of production of triplo-C have proved on test to have one C chromosome deficient for the color factor.

A number of other exceptional types of progeny have been secured; but since their constitution has not been definitely determined, further comments appear uncalled for at this time. As a matter of fact each of them presents an independent problem of considerable complexity, and many more of them appear than we have been able to analyze.

## DISCUSSION

The foregoing account demonstrates the feasibility of establishing monosomic types in *Tabacum* and of employing them for locating factors in specific chromosomes. None of the tests has been conducted on a large scale; one small culture is first grown and repetitions are made only if the results are in doubt. Obviously one of the advantages of the system is its ability to yield pertinent evidence in small cultures. The conduct of the experiments, however, reveals some of the limitations to successful employment of the method. Most of the populations must be grown from analytical crosses; consequently species like wheat, in which procurement of sufficient seed by hybridization involves an inordinate amount of effort, would scarcely be suitable subjects for such investigations, even if, as appears almost certain, a complete collection of monosomic types could be established in them. The method is obviously limited to those species in which transmissible monosomics may be established, very likely limited therefore exclusively to polyploid species, although we should not be surprised to find that monosomics of basic species, so far recorded as non-transmissible, may on more extensive trial exhibit a low rate of transmission.

Even with *Tabacum*, which is so highly favorable a subject for hybridization, location of a factor is still a laborious task, requiring tests with 24 monosomic types. We have therefore sought to devise a method which would reduce the amount of labor necessary for this purpose. It is possible, now that we have become familiar with the features of the 24 monosomics, that the asynaptic type, pale-sterile, may be used for the purpose. A cross of pale-sterile with a simple recessive gives in all cases so far tried a few recessive-type plants in the progeny. These plants should, at least normally, be monosomic for the chromosome in which the recessive factor is located. Single plants are often difficult to identify, particularly, since in this case plants of the immediate progeny from pale-sterile are likely to have a somewhat complicated cytological constitution, but a further backcross of these recessive plants to normal should lead to isolation and identification of the monosomic in the next succeeding generation.

This modification of procedure would substitute two progenies for the 24 necessary in direct use of the monosomic types; but it could only be used for location of simple recessives, which unfortunately appear to be scarce in *Tabacum*.

Employment of the method immediately suggests other problems to which it may be applied. Thus if duplicate genes are truly duplicate and have the evolutionary significance which has been ascribed to them, determination of their locations should provide a method of establishing specific cross homologies between chromosomes of the *tomentosa* and *sylvestris* subgenomes of *Tabacum*. If we can trust the meager evidence thus far available, the hairy-filament analysis demonstrates a cross homology between chromosomes A and O; and that of yellow-burley between B and O. Thus the first results appear to indicate that cross homology between the two subgenomes is segmental rather than chromosomal.

This consideration of duplicate genes in turn raises the question as to why some of our available simple recessives, particularly mammoth, pale-sterile, white-seedling, and yellowish-green, are not also governed by duplicate genes. Certainly the two species which are presumed to have contributed to the ancestry of *Tabacum* must both have been normal as respects such characters, and as a consequence the original raw amphidiploid must have had duplicate genes for them. Now that we are in possession of raw amphidiploids equivalent to *Tabacum*, such as 4n-*sylvestris-tomentosiformis*, it should be possible to re-establish the duplicate gene constitution of *Tabacum* with respect to such characters and thus to enlarge the possibilities of exploiting this method of determining specific cross homologies.

A fascinating system of analysis based upon employment of monosomics unfortunately has been rather disappointing in practice, owing to the complex genetic basis of most of the character differences in *Tabacum*. Consider a variety and the problem of analyzing its total differences from the chosen standard variety. Crosses of the variety with each of the monosomic representatives of the standard variety should immediately reveal in $F_1$ any simple recessive characters which distinguish the two varieties and should at the same time locate the factors in the chromosomes. A second backcross to the monosomic types should disclose the existence of dominant factors in the various chromosomes. Since theoretically entire chromosomes may be transferred unaltered by repeated backcrossing to the standard variety, it would seem possible to handle effectively even relatively difficult problems of size inheritance and other types of quantitative differences by this method. We have also occasionally found the method useful in unmasking hidden characters, as for filament color, as previously noted.

Monosomic analysis may also be adapted to determination of chromosomal dislocations. In a recent paper MALLAH (1943) has shown that a number of *Tabacum* varieties exhibit translocations relative to Purpurea. If such varieties be crossed with the Purpurea monosomics, cytological studies of the $F_1$ monosomics should reveal which specific chromosomes have been altered by trans-

location. For if one of the chromosomes is subtracted from a ring of four, obviously it becomes reduced to a chain of three, which is the condition to be expected in those $F_1$ types which are monosomic for chromosomes which have participated in the translocation.

## SUMMARY

The twenty-four monosomic types of *Tabacum* differ from normal and from one another in a specific ensemble of quantitative morphological features. Given a sufficiently uniform genetic background, they may be classified accurately on the basis of their morphology.

Monosomics may be established by design in a number of ways. Crosses of the asynaptic type, pale-sterile, with simple recessives permit direct production of types monosomic for the specific chromosomes which bear the recessive genes.

Thirty-five chromosome *sylvestris-Tabacum* counterparts also differ from normal *sylvestris-Tabacum* hybrids and from one another in a specific ensemble of morphological features, which may include qualitative as well as quantitative differences.

Study of the monosomes at MI disclose a wide range in size, as well as certain features of shape, which may aid in discrimination of types in some instances.

Variations in meiotic behavior include association of the monosome with a bivalent to form a trivalent, apparently largely limited to haplo-D and haplo-S; and promotion of non-conjunctional behavior in bivalents, the extent of which is apparently a specific feature of certain monosomic types.

On the basis of association of chromosomes in 35-chromosome *sylvestris-Tabacum* hybrids, monosomics may be classified into two groups, according as they are monosomic for chromosomes of the *tomentosa* or *sylvestris* subgenomes of *Tabacum*.

Pollen samples of monosomics exhibit specific differences, dependent upon the character and degree of development of the 23-chromosome microspores, which apparently uniformly constitute about 80 percent of the total number of microspores.

Ovular abortion rates of monosomics may reach a high level of about 80 percent as with pollen samples, and they are also characteristic for specific types.

Seed production rates for the monosomics are mostly characteristically depressed, especially in those which have high rates of ovular abortion.

Ovular transmission rates of the monosomics range from five to 80 percent. Monosomics which exhibit high ovular abortion rates show strongly depressed transmission values, but they still exhibit some transmission.

Studies of association in transmission between monosomics and Mendelian characters have led to location of 18 genes in nine chromosomes.

On the basis of location of genes for the duplicate factors concerned with hairy-filament and yellow-burley, a segmental cross homology is suggested between the A and O and the B and O chromosomes.

An oscillating relation exists as respects sporadic production of trisomic counterparts by monosomics, or monosomic counterparts by trisomics.

Attention is called to the possible value of monosomics in analysis of chromosomal dislocations as well as in determining the genetic basis of Mendelian characters.

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ADDENDUM

The reader's attention is called to the article by E. R. SEARS, Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics **29**: 232–246, which appeared while this paper was in press.

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# THE RELATION OF HOMOZYGOUS DEFICIENCIES TO MUTATIONS AND ALLELIC SERIES IN MAIZE

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IN PREVIOUS investigations (McCLINTOCK 1938, 1941b), the author has presented evidence that homozygous minute deficiencies of specific regions of chromosomes in maize are responsible for the appearance of readily recognizable modified phenotypes. These phenotypes resemble recessive mutations in their expression. One of them exactly simulated and was allelic to a known recessive mutant (*bm1*). The evidence obtained from these investigations strongly suggests that one type of mutation process in maize is induced by loss of a minute segment of a chromosome which, when homozygous, produces a distinct phenotypic expression. The present investigation both supports and elaborates this contention in the following way. A particular minute segment was lost from the tip of the short arm of chromosome 9. In plants that were heterozygous for the deficient chromosome, the gametophytes and gametes possessing the deficient chromosome were completely functional. Upon self-pollination of such plants, normal appearing kernels were obtained that were homozygous for the deficiency. The homozygous deficient seedlings arising from these kernels were specifically modified in their phenotypic expression. They were pale-yellow. The pale-yellow mutant, although caused by a homozygous deficiency, is comparable in its genetic behavior to any typical recessive mutant. Its mendelian ratio, its "locus" in the chromosome and its linkage with other known mutants in the chromosome are strictly orthodox. If the presence of the deficiency were not known, the mutation would receive the same consideration as a "gene mutation." This relationship between deficiency and mutation may be elaborated further. This same segment plus an additional adjacent segment was removed from the chromosome. Even when this longer deficiency is present, the male and female gametophytes and gametes are viable and functional. Thus, individuals homozygous for this deficiency may be obtained. These individuals, in turn, show a phenotypic modification (white seedling) distinguishable from that produced by the shorter deficiency. When the two deficient chromosomes are combined in a zygote, the resulting seedling shows the pale-yellow phenotype associated with the shorter of the two deficiencies. In other words, the two mutants are allelic. The mutant produced by the shorter deficiency is dominant over the mutant produced by the longer deficiency. This might be expected, for the individuals possessing these two deficient chromosomes are homozygous deficient for only the shorter of the two deficiencies, that is the deficiency which produces the pale-yellow phenotype. Combinations of these two deficiency mutants with the previously known recessive mutant *yg2* (yellow-green plants; mutant located near the tip of the short arm of chromosome 9) have shown that *yg2* is allelic to and dominant

over the mutant produced by the longer deficiency (the white seedling mutant) but is non-allelic to the mutant produced by the shorter deficiency (the pale-yellow mutant). It is the purpose of this paper to show that the pale-yellow and white mutants are caused by specific homozygous deficiencies and to clarify the seemingly anomalous allelic relationships.

The method which produces these specific deficiencies is relatively simple. Thus, similar deficiencies and consequently similar mutations may be independently and repeatedly obtained.

## THE METHOD OF OBTAINING TERMINAL DEFICIENCIES OF THE SHORT ARM OF CHROMOSOME 9

The deficiency mutants pale-yellow and white seedlings are associated with losses of terminal segments of the short arm of chromosome 9. The method by which terminal deficiencies arise has been described elsewhere (McCLINTOCK 1941a). It may be summarized briefly. Plants which possess a normal chromosome 9 and either a special rearrangement of segments of chromosome 9 or a chromosome 9 with a duplication of the short arm, can produce a dicentric chromatid at a meiotic prophase following specific types of crossing over between the two chromosomes 9. This dicentric chromatid produces a bridge configuration at one of the meiotic mitoses. Following breakage of this bridge, a chromosome 9 with a broken end will enter a spore nucleus. Depending upon where the break occurred within the bridge configuration, this chromosome 9 will be normal in chromatin constitution or will possess either a duplication or a deficiency of the short arm. All spores, except those with the very longest deficiencies, continue to develop. Because the two sister halves of the meiotically broken chromatid are fused at the position of previous breakage, a chromatin bridge is produced at the first spore anaphase as the two centromeres of the dicentric chromatid pass to opposite poles in the spindle figure. This bridge, in turn, will be broken and a newly broken chromatid will enter each telophase nucleus. The original meiotically broken chromosome 9 will continue this breakage-fusion-bridge cycle in the successive gametophytic divisions and also in the successive endosperm divisions whenever such a recently broken chromosome is introduced into the primary endosperm nucleus. However, when a chromosome 9 with such a recently broken end is introduced into the zygote by one of the gametes, the breakage-fusion-bridge cycle usually ceases in the young embryo. The broken end permanently heals and no longer participates in any fusions. The subsequent mitotic behavior of the chromosome 9 with a broken end is similar to that of any normal chromosome. In relatively infrequent cases, the chromosome with the broken end may continue the breakage-fusion-bridge cycle in the young embryo and even into the later developing sporophytic tissues.

When a zygote receives a normal chromosome 9 from one gamete and a recently broken chromosome 9 from the second gamete, the plants arising from these zygotes will have one normal chromosome 9 and, most frequently, one chromosome 9 with a broken but permanently healed end. The chromatin

constitution of this latter chromosome may be one of various types. This is because of its previous history of having been broken at meiosis and then having undergone the breakage-fusion-bridge cycle during the mitoses from meiosis to embryo formation. This chromosome may be normal in constitution or it may possess a duplication or a complex reduplication of segments of the short arm; or, terminal deficiencies or deficiencies plus duplications of segments may be the consequence of this behavior. Kernels which possess such a recently broken chromosome 9 may be identified by genetic means (for details, see McCLINTOCK 1941a). The chromatin constitution of the broken chromosome 9 in a plant arising from such a kernel can be determined by examination of pachytene configurations in this plant. The chromatin constitution of the broken chromosome 9 has been determined in over 500 plants which have arisen from such kernels. These plants were classified according to the observed modification in the constitution of the short arm of the broken chromosome 9. In all cases, the broken chromosome 9 contributed by one parent carried a dominant genetic marker in the short arm (*C*, aleurone color) whereas the normal chromosome 9 contributed by the other parent carried the recessive allele (*c*, colorless aleurone). The factor *C* is located in a normal chromosome 9 approximately one-third the distance from the end of the short arm. Although in a plant with unmodified chromosomes 9, more than 20 percent crossing over may occur between the locus of *C* and the end of the arm, a disturbed ratio of *C* to *c* could be expected following self-pollination of those plants which possessed a broken chromosome 9 with a decided modification of the short arm, such as a duplication or a deficiency. This is because the gametophytes with the modified chromosome carrying *C* might fail to function or might be reduced in functional capacity. Although all plants were self-pollinated to obtain this preliminary information on gametophytic functioning, our attention will be confined to (1) those that were classified as having received a broken chromosome 9 which is approximately normal, and (2) those which received a broken chromosome 9 with a deficiency. The extent of the deficiencies ranged from minute to extensive. All plants classified by pachytene studies as having a broken chromosome 9 with approximately a normal chromatin constitution gave normal ratios for aleurone color following self-pollination. Those classified as having received a chromosome 9 deficient for approximately one to six terminal chromomeres gave aleurone ratios suggesting that transmission of the deficient chromosome through the pollen did not occur, although transmissions through the female gametophyte were either normal or nearly so. (Six terminal chromomeres represent approximately the distal third of the short arm.) This was verified for each deficiency in later and more exacting tests. The majority of those plants classified as having a broken chromosome 9 with a very short terminal deficiency gave little or no evidence from the aleurone ratios of lack of functioning of either eggs or pollen grains carrying the deficient chromosome. It was presumed, therefore, that endosperms and embryos were being formed which were homozygous deficient for small terminal segments of the short arm of chromosome 9. From the

morphological appearance of endosperms or embryos, no distinction could be made, in many cases, between those kernels which were either normal or heterozygous for the deficiency and those which were homozygous for the deficiency.

To determine whether embryos with these homozygous deficiencies would germinate and produce viable seedlings, kernels from these self-pollinated ears were sown. For comparison, kernels from the self-pollinated ears of 30 plants classified as having a newly broken chromosome 9 with no deficiency were sown. All the seedlings arising from this latter group appeared normal. The plants arising from these seedlings were likewise normal in appearance. Some of these plants were examined at pachytene for their chromosome 9 constitutions. Two broken chromosomes 9 were present in some of these plants, indicating that no obvious phenotypic effects were being produced in plants that were homozygous for these broken chromosomes 9. In contrast, a segregation for seedling types occurred in the progeny of self-pollinated plants heterozygous for some of the small deficiencies. The modified seedlings in any one culture were either all pale-yellow or all white and in each culture, the ratio of these seedlings to the normal seedlings suggested a simple recessive mutation. In each case, linkage of the modified seedling type with the dominant aleurone factor *C* was clearly evident, indicating that the seedling character was associated with the deficient chromosome 9. This evidence suggested that the mutant seedlings, pale-yellow and white, might be produced when the chromosome complement was deficient for a small terminal segment of the short arm of chromosome 9. The following two sections of this paper will elaborate the methods used to verify this association. Seven independent cultures segregating pale-yellow seedlings and six independent cultures segregating white seedlings were selected for intensive study.

## THE PALE-YELLOW SEEDLING MUTANTS

The pale-yellow seedlings in all seven cultures were very much alike in appearance. The seedlings appear to be normal in morphological characters and in growth rates. Although chlorophyll is present in the coleoptile, which is light green in color, the leaves show only a light yellowish color. These seedlings die following depletion of essential nutritive reserves in the kernels. The seven independently arising pale-yellow mutants will be referred to as *pyd* 1 to 7, respectively. (This symbolization implies a pale-yellow phenotype, produced by a deficiency.)

From a purely genetic standpoint, the *pyd* mutants may be treated as any other recessive mutant in maize. Typical ratios of 3 normal green seedlings to 1 pale-yellow seedling appear in the selfed progeny of plants heterozygous for any one of the *pyd* mutants (table 1). Linkage with *C* is shown in table 2. Linkage with *yg2* (yellow-green plants), which is known to be located near the end of the short arm of chromosome 9 (CREIGHTON 1934, MCCLINTOCK 1941a), must be very close; tests of 115 chromosomes derived from plants which carried *Pyd yg2* in one chromosome and *pyd Yg2* in the homologous chromosome gave

TABLE 1

*The numbers of green and pale-yellow seedlings which appeared in the progenies of self-pollinated plants heterozygous for a deficient chromosome 9*

| SOURCE OF THE DEFICIENT CHROMOSOME | GREEN SEEDLINGS | PALE-YELLOW SEEDLINGS |
|---|---|---|
| *pyd*1 | 1411 | 445 |
| *pyd*2 | 3404 | 1148 |
| *pyd*3 | 1214 | 408 |
| *pyd*4 | 747 | 253 |
| *pyd*5 | 1565 | 545 |
| *pyd*6 | 1114 | 400 |
| *pyd*7 | 1910 | 636 |

no chromosomes with *Pyd Yg*2 or *pyd yg*2. The reported amount of crossing over between *yg*2 and *C* is approximately 19 percent (EMERSON, BEADLE and FRASER 1935). Estimates from the $F_2$ ratios of table 2 show that the amount of crossing over between *pyd* and *C* is similar to that between *yg*2 and *C*, although considerable variation between the *pyd* cultures is obvious. This variation is not considered significant since wide variations occurred between individual progenies within each *pyd* culture. On purely genetic evidence alone, the *pyd* mutants would be located near the tip of the short arm of chromosome 9.

TABLE 2

*$F_2$ progenies showing linkage of the pale-yellow phenotype with C. Constitution of the $F_1$: pyd C/Pyd c*

| | *C* KERNELS | | | | *c* KERNELS | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | SEEDLINGS | | | | SEEDLINGS | | |
| *pyd* CULTURE | NUMBER PLANTED | % GERMINATED | GREEN | PALE-YELLOW | NUMBER PLANTED | % GERMINATED | GREEN | PALE-YELLOW | % RECOMBINATION |
| *pyd*1 | 991 | 97.8 | 654 | 316 | 361 | 96.1 | 338 | 9 | 16 |
| *pyd*2 | 1151 | 95.8 | 739 | 364 | 418 | 90.9* | 366 | 14 | 20 |
| *pyd*3 | 1261 | 99.2 | 851 | 401 | 376 | 98.1 | 363 | 7 | 14 |
| *pyd*4 | 287 | 98.2 | 183 | 99 | 101 | 73.2* | 73 | 1 | 11 |
| *pyd*5 | 1202 | 98.6 | 775 | 411 | 412 | 95.4 | 384 | 9 | 15 |
| *pyd*6 | 719 | 96.9 | 476 | 221 | 228 | 86.4* | 195 | 2 | 11 |
| *pyd*7 | 776 | 95.8 | 504 | 240 | 284 | 94.7 | 260 | 9 | 18 |

* The mutant *sh* (shrunken endosperm) closely linked with *c* was segregating in some of these cultures. Lowered germination rates are often encountered when the kernels are homozygous *sh*.

Pachytene examinations of plants heterozygous for any one of the *pyd* mutants were likewise heterozygous for their chromosome 9 constitutions. In all cases, a normal chromosome 9 and a chromosome 9 with a deficiency of a minute terminal segment of the short arm were present. The short arm of a

normal chromosome 9 usually terminates in a knob composed of heterochromatin. This knob is joined to the first distinct chromomere by a relatively thin strand of stainable chromatin (see diagram a, fig. 2). The knob and this thin strand of chromatin are missing in the deficient chromosome of all plants heterozygous for a *pyd* mutant (see diagram c, fig. 2). It has been determined that plants which are homozygous deficient for only the knob are quite normal in appearance. Thus, the effective chromatin loss, associated with a mutation to *pyd*, is presumably confined to the segment which joins the knob and the first distinct chromomere (or to a particular minute region within this segment; see the Discussion). For each case, the exact extent of the deficiency could not be stated with certainty. The segments being examined are too small for such microscopic resolution. Whether a specific *pyd* deficiency includes a minute segment of the first terminal chromomere or whether a minute proximal segment of the strand joining this chromomere with the knob is present could not be determined. However, in all *pyd* cultures, there is no question of the presence of a terminal deficiency which includes most of the strand joining the first chromomere with the knob.

If the homozygous deficiency were responsible for the pale-yellow character, the surviving green seedlings in the progeny of a selfed heterozygous plant would be either homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9. Cytological examination of the chromosome 9 constitutions of the surviving green plants showed only these two types. In turn,

TABLE 3

*The cytologically determined chromosome 9 constitutions of the green plants of the various pyd cultures together with the results of tests for the presence or absence of the pyd mutants in each plant**

| *pyd* CULTURE | CHROMOSOME 9 CONSTITUTION OF TESTED PLANTS | | | | | |
|---|---|---|---|---|---|---|
| | TWO NORMAL CHROMOSOMES 9 | | | ONE NORMAL AND ONE DEFICIENT CHROMOSOME 9 | | |
| | NUMBER OF PLANTS EXAMINED | SEGREGATED *pyd* | DID NOT SEGREGATE *pyd* | NUMBER OF PLANTS EXAMINED | SEGREGATED *pyd* | DID NOT SEGREGATE *pyd* |
| *pyd*1 | 2 | 0 | 2 | 15 | 15 | 0 |
| *pyd*2 | 5 | 0 | 5 | 13 | 13 | 0 |
| *pyd*3 | 10 | 0 | 10 | 14 | 14 | 0 |
| *pyd*4 | 6 | 0 | 6 | 14 | 14 | 0 |
| *pyd*5 | 4 | 0 | 4 | 18 | 18 | 0 |
| *pyd*6 | 1 | 0 | 1 | 17 | 17 | 0 |
| *pyd*7 | 6 | 0 | 6 | 26 | 26 | 0 |
| Totals | 34 | 0 | 34 | 117 | 117 | 0 |

* Presence of the *pyd* mutant detected by one or more of the following methods: selfing, crosses to plants heterozygous for long terminal deficiencies of the short arm of chromosome 9, sib-crosses and intercrosses to various plants heterozygous for *pyd* or *wd* mutants (see text for elaboration of these methods).

if these latter plants are appropriately tested for the presence of the *pyd* mutant in one of their chromosomes 9, only the plants heterozygous for the deficiency should give rise to pale-yellow seedlings whereas those having two normal chromosomes 9 should not segregate any pale-yellow seedlings. This was found to be true in all subsequent progenies of cultures carrying the pale-yellow mutants. This evidence is summarized in table 3. Among the 151 plants cytologically examined, the 34 which possessed two normal chromosomes 9 gave rise only to normal green seedlings whereas all of the 117 plants which possessed a normal and a deficient chromosome 9 segregated pale-yellow seedlings.

To obtain further evidence for the association of the pale-yellow phenotype with a homozygous deficient condition, pollen from heterozygous deficient plants of each of the seven *pyd* cultures was placed upon silks of plants heterozygous for only female transmissible terminal deficiencies of the short arm of chromosome 9. These terminal deficiencies ranged in length from one chromomere to six chromomeres. If *pyd* were associated with a minute, terminal, male and female transmissible deficiency, pale-yellow seedlings should appear in the progeny of all such crosses following zygotic combinations of the two deficient chromosomes 9. In no case would the deficient chromosome contributed by the female parent cover the deficiency in the chromosome contributed by the male parent. This proved to be true (table 4).

The similarity in phenotypic appearance and location in the chromosome, as shown by linkage relations with *yg2* and *C*, of all seven independently arising

TABLE 4

*Phenotypic appearance of plants with the short terminal deficiency of the pyd and wd cultures and a longer terminal deficiency; py represents pale-yellow seedlings, w represents white seedlings*

| SOURCE OF DEFICIENT CHROMOSOME FROM ♂ PARENT | APPROXIMATE EXTENT OF TERMINAL DEFICIENCY OF THE SHORT ARM OF THE CHROMOSOME 9 CONTRIBUTED BY THE FEMALE PARENT | | | | |
|---|---|---|---|---|---|
| | 1 CHROMOMERE | 2 CHROMOMERES | 3 CHROMOMERES | 4 CHROMOMERES | 6 CHROMOMERES |
| *pyd1* | py | | py | | py |
| *pyd2* | | py | | | |
| *pyd3* | py | py | | py | py |
| *pyd4* | py | py | | py | py |
| *pyd5* | py | py | | | py |
| *pyd6* | py | py | | | py |
| *pyd7* | | py | | py | py |
| *wd1* | | w | w | w | w |
| *wd2* | w | w | w | w | w |
| *wd3* | w | w | w | w | w |
| *wd4* | w | w | | w | w |
| *wd5* | w | | | w | w |
| *wd6* | w | | | | w |

*pyd* mutants, together with a similar extent of deficiency in the chromosome 9 associated with each mutant, suggested that all seven *pyd* mutants were the expression of one and the same causal condition. If this were so, then combinations of any two of the seven *pyd* mutants should produce the pale-yellow phenotype. Intercrosses between heterozygous deficient plants of all seven cultures were made. Pale-yellow seedlings segregated in the expected ratios in the

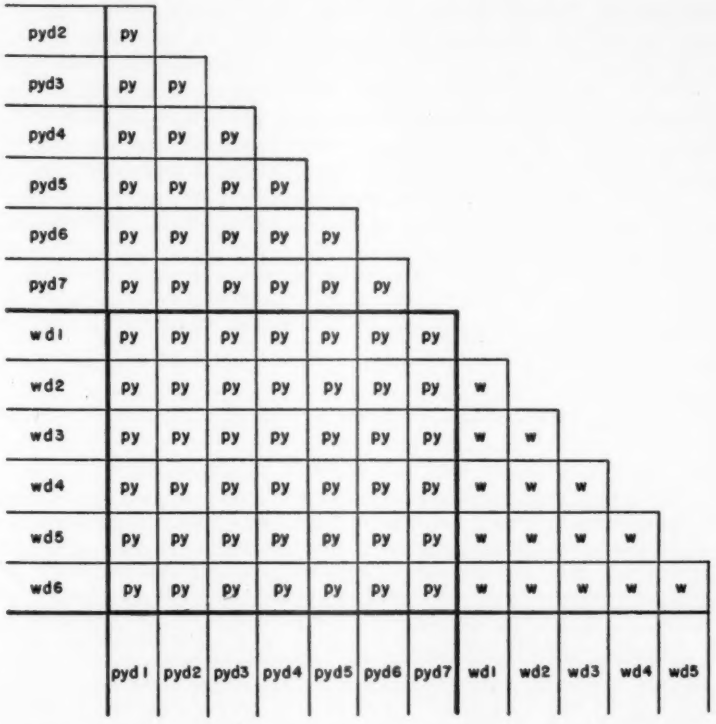

pyd2 py
pyd3 py py
pyd4 py py py
pyd5 py py py py
pyd6 py py py py py
pyd7 py py py py py py
wd1 py py py py py py py
wd2 py py py py py py py w
wd3 py py py py py py py w w
wd4 py py py py py py py w w w
wd5 py py py py py py py w w w w
wd6 py py py py py py py w w w w w
pyd1 pyd2 pyd3 pyd4 pyd5 pyd6 pyd7 wd1 wd2 wd3 wd4 wd5

FIG. 1.—The phenotypic appearance of seedlings following combinations of all seven *pyd* mutants (upper triangle), of all six *wd* mutants (triangle to lower right) and of all seven *pyd* mutants with all six *wd* mutants (central rectangle). The symbols py and w in the small squares represent pale-yellow and white seedling phenotypes, respectively.

progeny of all 21 possible combinations (fig. 1). For economy of space, the ratios of green to pale-yellow seedlings in the progeny of the 21 combinations have not been included in tabular form. However, all gave typical 3:1 ratios. These crosses established the iso-allelic if not identical nature of all seven *pyd* mutants. (Iso-alleles are defined by STERN and SCHAEFFER (1943) as alleles indistinguishable except by special tests.)

### THE WHITE SEEDLING MUTANTS

The six white seedling mutants are readily distinguishable from the pale-yellow mutants. The coleoptile in some cultures is very slightly tinged with yellow color whereas in other cultures it is chalk-white. The leaves are either chalk-white or slightly tinged with a very faint yellow color. Although the general morphological form of these white seedlings appears to be normal, they are always smaller than their sister green seedlings of the same age. The six white seedling mutants will be referred to as *wd*1 to *wd*6, respectively. This symbolization refers to the white phenotype produced by a deficiency.

The plants which segregate white seedlings in the six *wd* cultures are heterozygous for a terminal deficiency of the short arm of chromosome 9. These deficiencies are longer than those associated with the *pyd* mutants. They include not only the knob and the chromatin thread connecting the knob with the first distinct chromomere, as in the *pyd* mutants, but in addition a part of the first distinct chromomere is missing (see diagram d, fig. 2). In each case, it was not possible to determine the exact amount of terminal chromatin that was missing. However, the best preparations indicate that the deficiencies which cause the *wd* mutants extend to about the middle of the first chromomere.

The white seedlings in the progeny of self-pollinated heterozygous deficient plants die following depletion of essential nutritive reserves in the kernels. Cytological examination at pachytene of the chromatin constitution of the chromosomes 9 were confined, therefore, to the surviving green plants. Like

TABLE 5

*The cytologically determined chromosome 9 constitutions of the green plants of the various wd cultures together with the results of tests for the presence or absence of the wd mutant in each plant**

| *wd* CULTURE | CHROMOSOME 9 CONSTITUTION OF TESTED PLANTS | | | | | |
|---|---|---|---|---|---|---|
| | TWO NORMAL CHROMOSOMES 9 | | | ONE NORMAL AND ONE DEFICIENT CHROMOSOME 9 | | |
| | NUMBER OF PLANTS EXAMINED | SEGREGATED *wd* | DID NOT SEGREGATE *wd* | NUMBER OF PLANTS EXAMINED | SEGREGATED *wd* | DID NOT SEGREGATE *wd* |
| *wd*1 | 3 | 0 | 3 | 25 | 25 | 0 |
| *wd*2 | 14 | 0 | 14 | 10 | 10 | 0 |
| *wd*3 | 9 | 0 | 9 | 20 | 20 | 0 |
| *wd*4 | 9 | 0 | 9 | 17 | 17 | 0 |
| *wd*5 | 0 | — | — | 6 | 6 | 0 |
| *wd*6 | 1 | 0 | 1 | 15 | 15 | 0 |
| Totals | 36 | 0 | 36 | 93 | 93 | 0 |

* See footnote, table 3.

the *pyd* mutant cultures, only plants which were homozygous for normal chromosomes 9 or heterozygous for the deficient chromosome 9 were found. These plants, in turn, were tested for segregations of white seedlings in their progeny. None of the 36 examined plants with two normal chromosomes 9 gave rise to white seedlings, whereas all of the 93 examined plants which were heterozygous for the deficiency gave rise to white seedlings (table 5). This is to be expected if the white seedling phenotype is caused by the homozygous deficiency.

It was emphasized that the deficiencies associated with the pale-yellow phenotypes gave none of the usual genetic evidences of the presence of a deficiency. Except for the changed chlorophyll condition, all other examined tissues, homozygous for the deficiency, appeared to be normal. In contrast, the homozygous deficiency associated with the white seedling condition reflects the presence of a deficiency in several ways. In the first place, the transmission of the deficient chromosome through the pollen in competition with pollen carrying a normal chromosome 9, is reduced in two of the six *wd* mutants (*wd2* and *wd6*). Indications of this were apparent from the ratios of *C* to *c* obtained following self-pollinations of the heterozygous deficient plants (*C* carried by the deficient chromosome; *c* carried by the normal chromosome) and following backcrosses of these plants to normal plants homozygous for *c* (table 6, I and II). The aleurone ratios obtained from similar crosses involving the other four *wd* cultures did not suggest such selective reduction in pollen func-

TABLE 6

*I. Ratios of C to c following self-pollination of plants heterozygous for the deficient chromosomes 9 of the white-seedling cultures. C carried by the deficient chromosome, c carried by the normal chromosome.*

*II. Ratios of C to c obtained when the pollen of plants in I was placed upon silks of normal plants homozygous for c.*

| *wd* CULTURE | I | | II | |
|---|---|---|---|---|
| | *C* | *c* | *C* | *c* |
| *wd1* | 4296 | 1416 | 1126 | 1132 |
| *wd2* | 745 | 371 | 1455 | 1949 |
| *wd3* | 925 | 288 | 2781 | 2734 |
| *wd4* | 1761 | 588 | 1620 | 1549 |
| *wd5* | 387 | 276 | 1858 | 1781 |
| *wd6* | 2545 | 1146 | 2931 | 4883 |

tioning. More exact tests of the gametophytic transmissions (see page 491 and table 10) have shown that the transmission of the deficient chromosome through the female gametophyte is normal for all six white seedling-producing deficiencies and is normal through the pollen for *wd1*, 3, 4 and 5. However, in competition with normal pollen, the functioning of pollen carrying the deficient chromosome is reduced in the *wd2* and *wd6* cultures. The percentage reduction is approximately the same in each case. The pollen utilized had equal

numbers of normal and deficient grains. However, only one deficient pollen grain effected fertilization for every two normal grains. Although white seedlings appear when all six deficiencies are homozygous, it is to be expected from the method of origin that all six of these independently arising deficiencies need not be exactly alike in the extent of the deficiency (see Discussion). However, they all include the segment of chromatin which, when homozygous deficient, is responsible for the chlorophyll abnormality.

Examination of the kernels derived from self-pollinations of plants that are heterozygous for these deficiencies revealed another character which is consistent with a homozygous deficient condition. In all six cultures, some of the embryos had died during various stages of embryonic development. These embryos were shriveled and discolored and did not germinate. Kernels with such dead embryos could readily be classified. There was no consistency in the proportion of kernels with defective embryos among the self-pollinated ears

TABLE 7

*Segregation of defective embryos among the C and c kernels derived from self-pollination of plants heterozygous for the deficient chromosomes 9 of the white seedling cultures. C carried by the deficient chromosome, c carried by the normal chromosome.*

| *wd* CULTURE | *C* KERNELS | | *c* KERNELS | |
|---|---|---|---|---|
| | NORMAL EMBRYOS | DEFECTIVE EMBRYOS | NORMAL EMBRYOS | DEFECTIVE EMBRYOS |
| *wd*1 | 3553 | 743 | 1395 | 21 |
| *wd*2 | 710 | 35 | 367 | 4 |
| *wd*3 | 893 | 32 | 286 | 2 |
| *wd*4 | 1617 | 144 | 562 | 26* |
| *wd*5 | 784 | 53 | 275 | 1 |
| *wd*6 | 2245 | 300 | 1140 | 6 |

* Twenty-five of these kernels came from two of the six ears counted. Their cause is probably not related to the deficiency in chromosome 9.

within any white seedling culture. On some ears, no such embryos were present whereas on other ears they ranged from a few to approximately 25 percent of the embryos. Linkage of this defective embryo character with the mutant *C*, carried by the deficient chromosome 9, was obvious in all cases, suggesting that the cause of the defective embryo was associated with the deficiency (table 7).

In the progeny of self-pollinated heterozygous deficient plants, the typical $F_2$ ratio of 3 normal green seedlings to one white seedling is not always present. Sometimes there is a deficiency of the white seedling class. This would be expected if the homozygous deficiency causes death of some but not all of the developing embryos. Only those that survive during embryogeny could produce white seedlings. Lack of effective germination of some apparently living embryos which are homozygous deficient probably takes place for germination

rates were definitely reduced in some of these $F_2$ cultures. Also, within the *wd2* and *wd6* cultures, the reduced functioning of the pollen grains carrying the deficient chromosome 9 would tend to lower the percentage of homozygous deficient embryos and thus the proportion of white seedlings in the progeny. This latter factor, which reduces the expected proportion of white-seedlings in the $F_2$ progenies, is relatively constant whereas the former two factors are highly variable among the individual $F_2$ cultures.

Since wide variations in the proportion of normal to white seedlings occurred among the individual progeny tests within each white seedling culture, a composite table of these ratios for each of the white seedling cultures does not reveal the association of the reduction in the proportion of white seedlings with any one of the three mentioned causes. In a particular progeny, none, one, two or, in the *wd2* and *wd6* cultures, all three factors responsible for the reduc-

TABLE 8

*$F_2$ progenies showing linkage of the wd mutants with C. Constitution of $F_1$: Deficient chromosome 9 with C/normal chromosome 9 with c.*

| wd CULTURE | GOOD EMBRYOS | | | | | | | | DEFECTIVE EMBRYOS (NO GERMINATION) | | TOTAL SEEDLINGS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C KERNELS | | | | c KERNELS | | | | | | | |
| | NUMBER PLANTED | % GERMINATED | SEEDLINGS | | NUMBER PLANTED | % GERMINATED | SEEDLINGS | | C KERNELS | c KERNELS | | |
| | | | GREEN | WHITE | | | GREEN | WHITE | | | GREEN | WHITE |
| *wd1* | 1631 | 89.5 | 1283 | 177 | 639 | 96.2 | 615 | 0 | 339 | 9 | 1898 | 177 |
| *wd2* | 420 | 86.4 | 303 | 60 | 196 | 83.6† | 162 | 2 | 5 | 0 | 465 | 62 |
| *wd3* | 893 | 94.0 | 575 | 265 | 286 | 92.3 | 258 | 6 | 32 | 1 | 833 | 271 |
| *wd4* | 798 | 91.1 | 499 | 229 | 260 | 75.7† | 191 | 6 | 72 | 25* | 689 | 235 |
| *wd5* | 784 | 89.9 | 505 | 200 | 275 | 92.3 | 247 | 7 | 53 | 1 | 752 | 207 |
| *wd6* | 750 | 95.3 | 550 | 165 | 318 | 96.8 | 307 | 1 | 22 | 0 | 857 | 166 |

* See footnote, table 7.
† See footnote, table 2.

tion in the expected proportion of white seedlings may be active. This relationship is brought out in table 8 where the ratios for *C* and *c*, the proportion of defective embryos in each class, and the germination rates are considered. To illustrate how the three factors operate individually, the progenies from three selected ears in which only one factor was effectively operating in each case are given in table 9. In this table, a fourth progeny is added in which none of these factors was effectively operating. In this latter case, the expected ratio of 3 normal green to 1 white seedling is apparent.

The association of the white seedling character and the defective embryo condition with a homozygous deficient state can be verified by combining the deficient chromosomes of the white seedling cultures with the various female transmissible deficient chromosomes 9 given in table 4. Plants heterozygous for the female transmissible deficiencies of table 4 were crossed by plants heterozygous for the deficiencies of the six white seedling cultures. Some defective

TABLE 9

*$F_2$ progenies from three individual ears illustrating the three factors which materially reduce the expected proportion of white seedlings; together with the progeny from a fourth ear in which none of these factors was operating. Constitution of $F_1$: Deficient chromosome 9 with C/normal chromosome 9 with c.*

| FACTOR OPERATING | ALEURONE RATIO | | GOOD EMBRYOS | | | | | | | | DEFECTIVE EMBRYOS (NO GERMINATION) | | TOTAL SEEDLINGS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *C* KERNELS | | | | *c* KERNELS | | | | | | | |
| | | | NUMBER PLANTED | % GERMINATED | SEEDLINGS | | NUMBER PLANTED | % GERMINATED | SEEDLINGS | | | | | |
| | *C* | *c* | | | GREEN | WHITE | | | GREEN | WHITE | *C* | *c* | GREEN | WHITE |
| Reduced functioning of pollen with deficient chromosome (from *wd* 6 culture) | 258 | 101 | 257 | 98.4 | 193 | 60 | 101 | 99.0 | 100 | 0 | 1 | 0 | 293 | 60 |
| Defective embryos (from *wd*1 culture) | 317 | 119 | 226 | 96.9 | 210 | 9 | 118 | 97.4 | 115 | 0 | 91 | 1 | 315 | 9 |
| Poor germination (from *wd*1 culture) | 333 | 113 | 325 | 76.9 | 196 | 54 | 111 | 91.9 | 102 | 0 | 8 | 2 | 298 | 54 |
| No selective elimination (from *wd*3 culture) | 314 | 111 | 312 | 98.7 | 200 | 108 | 111 | 97.3 | 106 | 2 | 2 | 0 | 306 | 110 |

embryos, showing linkage with *C*, appeared in many of these crosses. In all cases, white seedlings likewise appeared in the progeny (table 4). These results are comparable to the selfed progeny of heterozygous deficient plants within the various white seedling cultures. This could be expected because the gametic combination of the two deficient chromosomes 9 would give rise to an individual which is homozygous deficient for only the short terminal deficiency of the white seedling cultures. It seems clear, then, that both the defective embryo and white seedling character are the consequence of the particular homozygous deficient state.

As stated previously, *yg2* is known to be located close to the end of the short arm of chromosome 9. Combinations of the deficient chromosomes 9 of the white seedling cultures with a normal chromosome 9 carrying *yg2* proved to be illuminating. It will be recalled that in the *wd* cultures the surviving green plants in the progeny of a self-pollinated heterozygous deficient plant are of two types: (1) those with two normal chromosomes 9 and (2) those with a normal and a deficient chromosome 9. When 15 plants of the former type were crossed by plants homozygous for *yg2*, the progeny from all 15 crosses gave only normal green plants. In contrast, when heterozygous deficient plants [(2) above] from all six *wd* cultures were crossed by plants homozygous for *yg2*, normal green plants and yellow-green plants appeared in the $F_1$ progeny. The

ratios in each case (table 10) were those expected if the green plants resulted from the zygotic combination of the normal chromosome 9 from the heterozygous parent with the *yg2* carrying chromosome and if the yellow-green phenotype resulted from the combination of the deficient chromosome with the *yg2* carrying chromosome. Six of these yellow-green plants were examined at pachytene for their chromosome 9 constitutions. All possessed one normal chromosome 9 and the deficient chromosome 9 of the *wd* cultures. These and four other yellow-green plants not examined cytologically, were appropriately tested for the presence of the white seedling mutant. The progeny tests revealed the presence of the *wd* mutant in all ten cases. In turn, none of the 22

TABLE 10

*Relative transmissions of the deficient and the normal chromosome through the ♀ and ♂ gametophytes of plants heterozygous for the various wd deficiencies. Tests made by reciprocal crosses of the heterozygous deficient plants to normal plants homozygous for yg2. The green plants in the progeny represent transmissions of the normal chromosome; the yellow-green plants represent transmissions of the deficient chromosome.*

| CULTURE | TRANSMISSIONS THROUGH THE ♀ GAMETOPHYTE | | TRANSMISSIONS THROUGH THE ♂ GAMETOPHYTE | |
|---|---|---|---|---|
| | GREEN | YELLOW-GREEN | GREEN | YELLOW-GREEN |
| *wd1* | 964 | 917 | 406 | 442 |
| *wd2* | 614 | 609 | 675 | 319 |
| *wd3* | 167 | 173 | 800 | 761 |
| *wd4* | 969 | 977 | 809 | 811 |
| *wd5* | 602 | 611 | 654 | 675 |
| *wd6* | 454 | 439 | 1350 | 679 |

cytologically examined green plants possessed a deficient chromosome 9 and none of the green plants segregated white seedlings following appropriate tests. These results indicated that from the point of view of phenotypic expression, the *wd* mutants are allelic and recessive to *yg2*.

The *yg2* factor was not present in either of the chromosomes 9 of the plants which gave rise to the broken chromosomes. Therefore, it cannot be concluded that *yg2* was present in the original broken chromosome unless a mutation to *yg2* occurred during the formation of the white seedling-producing deficiencies. As stated previously, the seven *pyd* mutants were not allelic to *yg2*. During the formation of these seven deficiencies responsible for *pyd*, no mutation to yellow-green occurred. The allelic relationships of *yg2* and *wd* is best explained by considering that the deficiencies causing the *wd* mutants are long enough to include the *Yg2* locus. The presence of the yellow-green plants in these crosses is, thus, the expression of a hemizygous condition, no complementary locus of *yg2* being present in the deficient chromosomes 9 of the *wd* cultures.

The allelic relations of *yg2* and the *wd* mutants allowed a convenient means of determining the transmissions of the deficient chromosomes through the

male and female gametophytes for each of the six deficiencies. To determine the transmissions through the female gametophyte, heterozygous deficient plants of the six *wd* cultures were pollinated by plants homozygous for *yg2*. To determine the transmissions through the male gametophytes (pollen grains), the reciprocal crosses were made. Because there are no viability factors connected with embryo development of the yellow-green phenotype, the ratio of green to yellow-green seedlings is a direct measure of the transmissions of the normal and the deficient chromosomes, respectively. The results are given in table 10. The deficient and the normal chromosomes are equally transmitted through the male and female gametophytes of cultures *wd*1, 3, 4 and 5. Equal transmissions occur through the female gametophytes of cultures *wd2* and *wd6* but the transmission of the deficient chromosome through the male gametophyte is definitely reduced. In both cases, approximately one-third instead of one-half of the progeny received the deficient chromosome. The allelic relationship of *yg2* and *wd* also allowed a determination to be made of the amount of crossing over which occurs between the mutant *C* and the end of the short arm of the deficient chromosome. Reciprocal crosses were made between normal chromosome 9 plants homozygous for *yg2* and *c* and heterozygous deficient plants carrying *Yg2* and *c* in their normal chromosome 9 and *C* in their deficient chromosome 9. The non-crossover chromatids would give rise to (1) colorless kernels, green plants and (2) colored kernels, yellow-green plants. The crossover chromatids would give rise to (1) colorless kernels, yellow-green plants and (2) colored kernels, green plants. Within each white seedling culture, wide variations in crossover percentages were found among the individual progenies. Considerably less variation occurred when the male was the heterogametic parent; the average for all six cultures was 15.2 percent, which is close to the 19 percent previously reported for *yg2* and *C*.

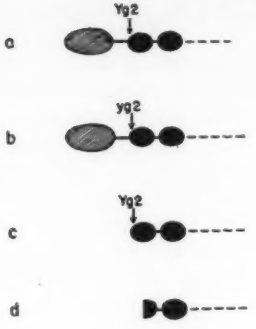
The similar appearance of the white seedling phenotypes, the presence of defective embryos, the allelic relations with *yg2* and the association with a terminal deficiency of all six white seedling mutants suggested that they might be either identical or iso-allelic. To determine this, intercrosses between heterozygous deficient plants of all six cultures were made. Both defective embryos and white seedlings showing linkage with *C* segregated in the $F_1$ progeny of all 15 combinations establishing, therefore, their iso-allelic if not identical nature (fig. 1).

The description so far given allows one to draw the following conclusions. The seedling mutant pale-yellow will appear whenever a plant is homozygous deficient for a small terminal segment composed of the knob and the chromatin strand joining the knob and the first distinct chromomere. All such mutants will be allelic if not identical. They will not be allelic to *yg2* but will be very closely linked with this locus. A white seedling mutant will appear whenever a plant is homozygous deficient for this same segment plus a particular part of the adjacent chromomere. All of these white seedling mutants will be either identical or allelic. In contrast to the pale-yellow mutants, all the white seedling mutants will be allelic and recessive to the mutant *yg2*. Following these

conclusions, one should expect that the combination of the deficient chromosome which produces pale-yellow seedlings and the deficient chromosome which produces white seedlings would give rise to the pale-yellow phenotype for these seedlings would be homozygous deficient for only the segment associated with the pale-yellow phenotype. To determine this, intercrosses of heterozygous deficient plants of all seven *pyd* cultures with heterozygous deficient plants of all six *wd* cultures were made. In all 42 combinations, pale-yellow seedlings segregated in the expected ratios in the $F_1$ progeny (fig. 1). Furthermore, there were no defective embryos regularly appearing in the progeny of these crosses. It is clear then, that the pale-yellow and white mutants are allelic and that the white mutants are recessive to the pale-yellow mutants.

### GRAPHIC INTERPRETATION OF THE ALLELIC RELATIONS OF *pyd*, *wd* AND *yg2*.

A consistent hypothesis can be formulated to account for the appearance of the pale-yellow and white seedling mutants and their allelic relations with each other and with *yg2*. This hypothesis considers that the phenotypes *pyd* and *wd* are due to homozygous deficiencies, as elaborated in the previous sections. Likewise, it is possible that *yg2* may be due to or simulated by a homozygous minute internal deficiency. Whether or not *yg2* is due to a homozygous deficiency or a true "gene" mutation is immaterial, however, in the explanation of the allelic relations of these three mutants. To facilitate this interpretation, a diagram, figure 2, has been constructed. The short arm of a normal chromosome 9 carrying *Yg2* and terminating in a knob is shown in **a**, figure 2; **b**, figure 2, represents a normal chromosome 9 carrying *yg2*. In **a** and **b** the arrow points to the locus of *Yg2* and *yg2* respectively. In **c**, a terminal segment is missing. This is the segment which, when homozygous deficient, is responsible for the pale-yellow mutant. It should be noted that this deficient segment does not include the *Yg2* locus. In **d**, a longer terminal segment is missing. It is the segment which, when homozygous deficient, produces the white seedling mutants. It should be noted that this deficiency includes the locus of *Yg2*. Below and to the left of the diagram is given the phenotypes appearing when a plant is homozygous for any one of these chromosomes. To the right are given the phenotypes produced following combinations of any two of these chromosomes. The normal chromosome 9 with *Yg2* (**a**, fig. 2) covers the recessive mutant *yg2* of **b**, and the deficiencies of both chromosomes **c** and **d**. Thus, only green seedlings arise following combinations of this chromosome with any one of the other three. The combination of **b** plus **c** gives rise to a green seedling because the *yg2* carrying chromosome covers the deficiency in chromosome **c** whereas the deficient chromosome **c** carries the dominant allele of *yg2*. The combination of **c** and **d** gives rise to a pale-yellow seedling because the residual homozygous deficiency is only that which produces the pale-yellow phenotype. In the combination **b** plus **d**, however, the seedling is yellow-green because the terminal deficiency in chromsome **d** is covered by chromosome **b** but chromosome **d** does not cover the *yg2* locus with *Yg2* because it is deficient for this locus.




| | Phenotype appearing when homozygous | | Phenotype appearing following combinations | |
|---|---|---|---|---|
| a+a | green seedling | a+b, c or d | green seedling |
| b+b | yellow-green seedling | b+c | green seedling |
| c+c | pale-yellow seedling | b+d | yellow-green seedling |
| d+d | white seedling | c+d | pale-yellow seedling |

FIG. 2.—**a.** Diagram of the chromatin organization of the end of the short arm of chromosome 9. The large hatched oval represents the terminal heterochromatic knob. This is followed by a thin chromatic segment which joins the first distinct chromomere with the knob. The small, solid ovals represent the two distal chromomeres. The arrow points to the locus of *Yg2*. **b.** Same as **a**, except that the chromosome carries the locus of *yg2* (arrow). **c.** The end of the short arm of a chromosome 9 deficient for the knob and the segment which joins the knob with the distal chromomere. The locus of *Yg2* is marked by the arrow. This deficiency, when homozygous, gives rise to the pale-yellow seedling phenotype. **d.** Slightly longer terminal deficiency than in **c**. The locus of *Yg2* has been lost. This deficiency, when homozygous, gives rise to the white-seedling phenotype.

The mutants *yg2*, *pyd* and *wd* give rise to peculiar allelic relationships which might be difficult to interpret were the cytology not known. With regard to dominance, there are two series of descending order: I, green→*pyd*→*wd* and II, green→*yg2*→*wd*. The white mutants are common to both series but the *pyd* mutants and *yg2* are not allelic.

### THE RATE OF PRODUCTION OF THE *pyd* AND *wd* MUTANTS BY RECENTLY BROKEN CHROMOSOMES 9

It was stated in the introduction that the mutants *pyd* and *wd* appeared repeatedly in the progeny of plants which had received a newly broken chromosome 9. As described earlier, each of the seven *pyd* mutants and each of the six *wd* mutants described in this paper arose independently from a chromosome 9 which was first broken at a meiotic anaphase. Large numbers of functional male gametes containing recently broken chromosomes 9 may be obtained by

special methods (McCLINTOCK 1943). Because of the breakage-fusion-bridge cycle which these meiotically broken chromosomes undergo in the succeeding gametophytic mitoses, the gametes carrying recently broken chromosomes 9 have various modifications in the constitution of the short arm (see page 480). To obtain some estimate of the proportion of functional male gametes which introduce into the embryo the deficiencies responsible for pale-yellow or white seedlings, the following experiment was performed. The silks of plants that were heterozygous for the longer terminal deficiencies of table 4 (that is, deficient for four or six terminal chromomeres) were pollinated by plants that are producing meiotically broken chromosomes 9. The gametophytes produced by the female parent are of two types, those possessing a normal chromosome 9 and those possessing a long terminal deficiency of the short arm of chromosome 9. Whenever male gametes with recently broken chromosomes 9 are delivered by pollen tubes to these female gametophytes, kernels with morphologically normal endosperms will be produced when the female gametophyte possesses the normal chromosome 9. In contrast, aberrant endosperms will be produced when the female gametophyte possesses the deficient chromosome 9. This is due to the subsequent behavior of the broken chromosome 9 delivered to the endosperm by the male parent. It undergoes the breakage-fusion-bridge cycle (McCLINTOCK 1941a) during endosperm development. This process brings about deletions of segments of the short arm of this chromosome 9 in some cells during endosperm development. Since the chromosomes 9 delivered by the female parent are already deficient for a long terminal segment, the telophase nucleus which receives this newly broken chromosome with a terminal deficiency will be homozygous deficient for a segment of the short arm of chromosome 9. In these nuclei, the extent of the homozygous deficiency may range from minute to the full extent of the deficiency in the chromosomes 9 delivered by the female parent. All of these homozygous deficient cells are viable and capable of multiplication. Cells with the longer homozygous deficiencies produce sectors within the endosperm which are sufficiently aberrant to be readily recognizable (McCLINTOCK 1942). Thus, kernels receiving deficient chromosomes 9 from the female parent and a recently broken chromosome 9 from the male parent may be readily detected and selected from an ear. The embryos of these kernels will have the deficient chromosome delivered by the female parent and the newly broken chromosome delivered by the male parent with the exception of a few cases where hetero-fertilization may have occurred. The chromatid type of breakage-fusion-bridge cycle, which occurs in the gametophyte and endosperm tissues, usually does not occur in the sporophytic tissues. The broken end usually heals in the very young embryo and the broken chromosome is completely normal in its mitotic behavior from then on. If the healed broken chromosome 9 has at least a full genic complement of the short arm of chromosome 9, green seedlings should arise from the embryos of these kernels. If it has a short terminal deficiency either pale-yellow or white seedlings could appear because the cells would be homozygous deficient for the short terminal deficiency. If it has a terminal deficiency much beyond the ex-

tent of the *wd* mutants described in this paper, the embryos are expected to be inviable.

From the cross just described, 3287 seedlings were obtained from kernels classified as having received a deficient chromosome from the female parent and a newly broken chromosome from the male parent. Of these seedlings, 77 were pale-yellow and 48 were white. From these results it is concluded that among the viable zygotic combinations, one recently broken chromosome in every 26 had either a deficiency which produced *pyd* or a deficiency which produced *wd*.

These results, together with those already presented for the seven *pyd* and six *wd* mutants described in the previous sections of this paper, illustrate the repeated occurrence of phenotypically and genetically similar mutants. The described chromosomal breakage mechanism is, then, a "mutation inducing" process which "induces" the same mutation time and again.

## DISCUSSION

Evidence that some recessive mutations are the consequency of homozygous minute deficiencies has been accumulating in both Drosophila and maize. In Drosophila, the phenotypic characteristics of *y* (EPHRUSSI 1934; STERN 1935; MULLER 1935; DEMEREC 1936; DEMEREC and HOOVER 1936), *sc* (STURTEVANT and BEADLE 1936), *ac* (MULLER 1935), *rst*$^2$ (EMMENS 1937; PROKOFYEVA-BELGOVSKAYA 1939; PANSHIN 1941), *w* (PANSHIN 1938, 1941) and possibly *fa* (OLIVER 1937, 1938) in the X chromosome may appear when the + locus of these mutants are missing from the chromosome, that is, when the organism is homozygous deficient, in each case, for a particular minute segment of chromosome. In maize, the appearance of white seedlings as the consequence of a homozygous deficiency of the tip of the short arm of chromosome 9 was first observed by CREIGHTON (1937). This deficiency was internal in that only the proximal part of the knob was included in the deficiency. This deficiency was very minute and it probably included the same segment of chromatin that is responsible for the white seedling phenotypes described in this paper. This deficiency was male and female transmissible and produced white seedlings when homozygous. When combined with *yg2*, the yellow-green phenotype appeared, indicating that the locus of *Yg2* had been included in the deficiency. The genetic behavior of this cytologically similar deficiency duplicated the behavior of the deficiencies causing the white seedlings described in this paper. However, because this stock has been lost, a test for identity could not be made.

A series of recessive mutants associated with homozygous minute deficiencies confined within the limits of a few chromomeres adjacent to the centromere of the short arm of chromosome 5 in maize has been reported previously (MCCLINTOCK 1941b). One of these deficiencies resembled in all ways and was isoallelic if not identical to a previously known recessive mutant (*bm1*) which has been located within this segment. It seems reasonable to conclude that one form of mutation is related to loss of a particular minute segment of

chromatin or to the inactivation of this particular minute segment. The same character could appear following either condition. However, reverse mutation would not be anticipated following loss of a locus, whereas such a reverse mutation might occur following inactivation of a locus. The *y* locus in the X chromosome of Drosophila may illustrate this distinction. Some mutations to *y* may be the consequence of a minute chromatin loss. Other mutations to *y* may be due to inactivations for reversions from *y* to $y^+$ have been reported (JOHNSTON and WINCHESTER 1934; DUBININ and GOLDAT 1936).

With so few analysed cases available, it is difficult to ascertain the role that homozygous minute deficiencies or inactivations play in the whole mutation process. It seems reasonable to believe that they may play a large part in maize. Within the confines of four chromomeres adjacent to the centromere of the short arm of chromosome 5, six distinct non-allelic mutants, five of which were color mutants and one of which was a developmental mutant, were distinguished. All these mutants were associated with homozygous minute deficiencies. All were both male and female transmissible (MCCLINTOCK 1941b and unpublished). Again, in this paper, mutants associated with minute losses of chromatin have been described. These, too, are both male and female transmissible. Since a color change was the factor which made most of these mutants readily recognizable, it is reasonable to conclude that other mutants, not associated with color changes, are being produced as the consequency of homozygous minute deficiencies. There is no reason to believe that the two chromosome regions in maize which have been selected for study are exceptional samples of the whole chromosomal complement. Their selection was merely a matter of chance because of structural abnormalities that had happened to these chromosomes. It was these structural abnormalities that furnished the means for a study of homozygous minute deficiencies.

In this paper, it has been stated that the recessive mutants pale-yellow and white were due to progressive losses of chromatin. The *pyd* mutants appeared when the chromatin between the knob and the first distinct chromomere was missing and the *wd* mutants appeared when this segment plus an adjacent segment of the first chromomere was missing. The author does not believe that this indicates that the phenotypes pale-yellow and white are due to cumulative effects of the losses described. It is possible that the pale-yellow phenotype is related to loss of a particular locus in the proximal region of the segment which is missing; and that the white phenotype represents the effect of this particular loss plus loss of another particular locus in the adjacent chromomere or loss of only a single locus in this chromomere. The fact that other chromatin is also missing in each case may have little or no relation to the phenotypic expressions of pale-yellow or white. A suggestion that the particular phenotypic characters pale-yellow and white may be due to losses of specific loci rather then cumulative effects of a series of loci, may be seen in the differences between *wd*1, 3, 4, 5 and *wd*2 and 6 in the transmission of the deficient chromosome through the pollen. The four white seedling mutants in the former case have normal transmissions of the deficient chromosomes whereas the latter

two white seedling mutants have a reduced transmission through the pollen. It is possible that a slightly longer deficiency is present in *wd2* and 6. However, when homozygous, this added deficiency does not affect the expression of the white seedling phenotype. The white seedling mutants are semi-dwarfed. This reduced growth rate may be due either to a cumulative effect of various homozygous deficient loci or to a specific locus which is not related to the locus whose absence is responsible for the chlorophyll abnormality. Similarly, death of some of the homozygous deficient embryos in the white seedling cultures may be a reflection of the same phenomenon. Internal deficiencies of specific loci within this segment are required to differentiate between these alternatives. Cytologically, it might be difficult to identify such minute internal deficiencies. In this study, it was only because the segments were terminal that it was possible to analyse the extent of the minute deficiencies with any reasonable degree of certainty. If these deficiencies had been internal, a positive conclusion might not have been obtained. This is because, following homologous association of a normal and a deficient chromosome, the chromomeres adjacent to the internal deficiency might frequently be stretched and distorted during the preparation of the sporocytes for microscopic observation. The sporocytes in pachytene are gently pressed to flatten the chromosomes. When no structural heterozygosity is present, homologous chromomeres remain together during this process. If, however, a small internal deficiency were present in one chromosome, the corresponding non-deficient segment in the homologous chromosome might be subject to tension while being flattened. This tension could result in distortion of the form of the chromomeres adjacent to the deficiency in the deficient chromosomes. This would cause difficulty in the determination of the extent of a very small internal deficiency. When the deficiency is terminal, the free ends of the synapsed chromosomes are not subject to this type of distortion so that small terminal deficiencies may be satisfactorily analysed.

In Drosophila, mutations may be associated with homozygous deficiencies, with duplications, with various "position effects," or dominant mutants may appear when various regions of the chromosome are hemizygous (the Minutes, Notchs, etc.). These mutants are not considered as having arisen solely from modifications of a specific locus—a "genic change." When a mutation arises which is not associated with a visible change in a chromosome, it is not possible with our present methods to know whether a minute deficiency or duplication is present, whether inactivation or a molecular change in a so-called gene has occurred, or whether structural alterations giving "position effects" have occurred. This applies to the majority of mutants that have been studied. From the accumulating evidence in maize and Drosophila, it is conceivable that many of these mutants are not caused by "genic changes," if this is construed to mean a molecular change in an isolated unit. It appears to the author that the interchangeable use of the terms "mutant" and "gene" should be avoided in order not to prejudice ones thinking of genic action.

Just as mutations are not always caused by "genic changes" at a specific

locus, so are alleles not always caused by "genic changes" at a specific locus. In this paper, pale-yellow and white behave as alleles and white and yellow-green behave as alleles but pale-yellow and yellow-green do not behave as alleles.The interpretation given in this paper adequately accounts for these allelic expressions. It is not necessary to invoke a "genic change." Whether or not allelic expressions for specific mutants will occur may depend upon the particular modification which gave rise to the mutation in each case. It is possible that the *pyd* mutant is due to a loss of a specific locus and that *wd* is due to loss of another nearby but independent locus, and also *yg2* may be caused by loss of still another independent locus. If this is true, it should be possible to obtain a chromosome with only the *Pyd* locus missing and also a chromosome with only the *Wd* locus missing. No allelic expressions of *pyd* and *wd* or of *yg2* and *wd* would be anticipated following combinations of these chromosomes. It is only because the *pyd* and *wd* mutants described in this paper have relatively large segments of chromatin missing that we are certain to obtain residual deficiencies and thus allelic expressions following combinations of these deficient chromosomes. Thus, whether or not two or more mutants will show allelic relationships may depend upon the particular modification which gave rise to the mutant. Following some modifications, two mutants, *a* and *b*, may show allelic expressions. If, following another modification, the *a* mutant phenotype arises again, this mutant may show an allelic expression with the original *a* mutant but need not show an allelic expression with *b*. Alleles in the Truncate series, the vestigial series and the facet-Notch series in Drosophila may illustrate such variations in allelic expressions.

In Drosophila, the allelic expression of the *sc* (scute) series (for extensive literature citations see GOLDSCHMIDT (1938), resembles the allelic expressions of *pyd*, *wd* and *yg2*. Overlapping and residual effects follow combinations of specific alleles. This similarity in allelic expression, however, does not presuppose a similarity in cause. The mutants *lz*$^{s}$ (spectacle) and *lz*$^{g}$ (glassy) behave as alleles but this allelic expression disappears following specific types of crossing over (OLIVER 1940, 1941). Likewise, in Drosophila, the mutants *S* (dominant star) and *ast* (recessive asteroid), which are 0.02 crossover units apart behave as alleles when carried by opposite chromosomes but this allelic expression disappears when both mutants are carried by the same chromosome (LEWIS 1941, 1942). Also, in Drosophila, a deficiency of a particular segment of a chromosome may give rise to a dominant (homozygous lethal) mutant which shows some resemblance to a recessive mutant whose locus is in the deficient segment. When the deficient chromosome and a chromosome with the recessive mutant are combined, the phenotypic expression may be exaggerated form of the recessive mutant. According to BRIDGES (MORGAN, BRIDGES and SCHULTZ 1938), these mutants are "pseudo-allelic." The term "pseudo-allelic" presupposes a knowledge of some special alteration which accompanies the expression of allelism. When this knowledge is not present no "pseudo" modifies the term "allelic." Various causal factors produce mutations and are responsible for allelic expressions. Mutants giving allelic expressions need not be "located"

at comparable positions in homologous chromosomes and they need not be inseparable by crossing-over. It has been the purpose of this paper to analyze one type of modification which gives rise to mutants that show allelic expressions.

The induction of mutations by various means (X-rays, neutrons, U.V. light, heat, age, moisture content of seeds, etc.) has occupied the attention of many geneticists and has proven highly effective. By none of these agents, however, has it been possible to control the particular mutation which will appear. To this list one can add the method described in this paper which involves the repeated occurrence of breaks that are confined within the limits of a single arm of a particular chromosome. Literally thousands of such newly arising broken chromosomes can be obtained with extraordinarily little effort. Since the chromosome arm involved is relatively short, there is a good chance that among a large number of such breakages, many will occur at approximately the same position. In other words, terminal deficiencies of approximately the same length could repeatedly be produced. It has been shown in this paper that the mutants *pyd* and *wd* are associated with such terminal deficiencies. Thus, the mutants *pyd* and *wd* should appear repeatedly in the progeny of plants receiving such newly broken chromosomes. That this is true, has been demonstrated. This broken chromosome method of mutation induction differs from the agents mentioned above in that it repeatedly produces the same mutants. In this respect, it simulates the behavior of the *Dt* mutant in maize which repeatedly induces mutations at a particular locus in another chromosome (RHOADES 1938). However, the mutation process in the two cases is altogether different.

## SUMMARY

A number of individuals were obtained possessing a normal chromosome 9 and a chromosome 9 whose short arm was deficient for a terminal segment of chromatin. In each plant, the deficient chromosome was introduced by one parental gamete following breakage of the short arm of chromosome 9 in the previous meiotic mitosis of this parent. The extent of these deficiencies ranged from minute to the full short arm. The smaller terminal deficiencies were both male and female transmissible. Self-pollination of plants heterozygous for these smaller terminal deficiencies gave rise to kernels with homozygous deficient endosperms and embryos. In any one progeny, the seedlings arising from these kernels were either pale-yellow or white.

Seven of these cultures which segregated pale-yellow seedlings were selected for study. In each case, it was determined that the pale-yellow phenotype was produced when the seedlings were homozygous deficient for a minute terminal segment. The seven pale-yellow mutants were comparable in all ways to typical recessive mutants. All seven independently arising pale-yellow mutants were allelic.

Six cultures which segregated white seedlings were selected for study. In all six cases, it was shown that the white seedling phenotype appeared when the seedlings were homozygous for the deficiency. All six white seedling mutants

were allelic. The terminal deficiencies producing the white phenotype are slightly longer than those producing the pale-yellow phenotype.

Intercrosses of the seven pale-yellow mutants with the six white mutants showed that the two types of mutants were allelic. The pale-yellow mutants were dominant to the white mutants. This could be expected, for the individuals possessing a pale-yellow producing deficiency and a white producing deficiency are homozygous deficient for only the shorter of the two deficiencies, that is, the deficiency which produces pale-yellow.

The seven pale-yellow mutants and the six white mutants were combined with a previously isolated recessive mutant yellow-green 2 (*yg2*) known to be located near the end of the short arm of chromosome 9. The seven pale-yellow mutants were not allelic to *yg2* but all six white mutants were allelic and recessive to *yg2*. The allelic expressions of pale-yellow and white, of white and *yg2* and the nonallelic expression of pale-yellow and *yg2* are readily interpretable if it is assumed that the longer deficiency, which produces the white phenotype, included the locus of *Yg2* whereas the shorter deficiency, which produces pale-yellow, does not extend to this locus.

The method of origin of these terminal deficiencies in the short arm of chromosome 9 is relatively simple. Large numbers of newly derived deficiencies may readily be obtained. Many of these should be of approximately the same length. Since the mutants pale-yellow and white are due to specific deficiencies, these same mutants should appear repeatedly in the progeny of individuals that receive these newly derived deficient chromosomes. Special tests, conducted to determine this, confirmed this expectation.

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